



Insights from the past

Retrospective monitoring of genetic variation in Atlantic cod (*Gadus morhua*)

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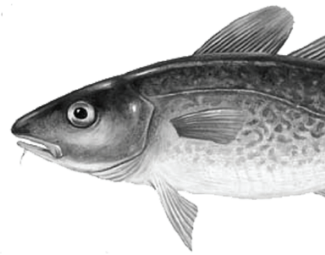
Insights from the past

Retrospective monitoring of genetic variation in Atlantic cod (*Gadus morhua*)

PhD Thesis



Written by Nina Overgaard Therkildsen
Defended 17 August 2012



Insights from the past

Retrospective monitoring of genetic variation in Atlantic cod (*Gadus morhua*)

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Cover photo: Atlantic cod otolith collected in Greenland 1954 (N. O. Therkildsen)

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Preface

This thesis was submitted in partial fulfillment of the requirements for the Doctor of Philosophy Degree (PhD) at the Technical University of Denmark (DTU). The presented research was conducted between June 2009 and June 2012, primarily at the National Institute of Aquatic Resources at the Technical University of Denmark (DTU Aqua) in Silkeborg under supervision of Professor Einar Eg Nielsen (main advisor) and Research Scientist Jakob Hemmer-Hansen (co-advisor). The PhD study also included a 1.5-month research visit at the Centre for Geogenetics at the Natural History Museum of Denmark and a 6-month research visit in the laboratory of Professor Stephen R. Palumbi at Hopkins Marine Stations, Stanford University, CA, USA.

The PhD study was funded by DTU, DTU Aqua, and the PhD program SLIP. Additional research funds were provided by the European Commission, as part of the Specific Targeted Research Project Fisheries-induced Evolution (FinE, contract number SSP-2006-044276) and the Danish Agency for Science, Technology and Innovation as part of the Greenland Climate Research Centre. Foreign research visits were funded through an EliteForsk travel grant from Danish Agency for Science, Technology and Innovation, for which I am very grateful.

The goal of the PhD research was to elucidate how natural populations of Atlantic cod (*Gadus morhua*) have reacted to natural and human-induced changes to their environment over the past century. We examined this through comparison of historical and contemporary DNA samples that provided a unique opportunity to retrospectively track genomic signatures of microevolution in recent time. The project has only been possible because of important contributions from many people. First and foremost, I would like to thank my primary advisor Einar Eg Nielsen for creating the opportunity to conduct this exciting research, for support and inspiration, insightful discussions, and for always helping to lift my focus from the details to the bigger picture. I continue to learn so much. Many thanks also to my co-advisor Jakob Hemmer-Hansen for all the support and for so generously sharing data and stimulating perspectives. Likewise, I am immensely grateful to Dorte Meldrup for teaching me everything I know about using pipettes, and for invaluable assistance and encouragement in the laboratory. The rest of the population genetics group in Silkeborg – Karen-Lise, Noor, Morten, Kristian, Sara, Thomas, and Dorte – has also been extremely supportive and helpful – thanks very much for creating such an engaging and caring work environment.

I would also like to express my sincere gratitude to Steve Palumbi at Stanford University and all the members of his research group for so warmly welcoming me for 6 very inspiring months in their company. The gratitude extends to the awesome Julie Stewart for hosting me and showing such incredible hospitality during my stay in California. Many thanks also to Tom Gilbert and Eske Willerslev at the Centre for GeoGenetics at the Natural History Museum in Copenhagen for generously providing access to their ancient DNA laboratories. Rob Ogden at Trace Network, as well as Richard Talbot and David Morrice at the Roslin Institute, have also been very helpful in assisting with SNP genotyping.

I would like to thank all my collaborators and colleagues who have contributed important ideas and support for my research. In particular, I want to acknowledge the much appreciated help from Doug Swain, Joanne Morgan, Ed Trippel, Mary S. Wisz, Peter Grønkjær, Rasmus Hedeholm, Anja Retzel, and members of the EU-funded FinE project. I am also grateful to all my fellow students for sharing good times and exciting discussions. Very special thanks go to Morten T. Limborg for always being there as my close companion during these past years of research- and life exploration, and for – together with his wonderful wife Maria – becoming my Silkeborg family.

This PhD is a culmination of a long education through which I have been privileged to work with a long list of extremely talented and inspiring teachers. I would like in particular to acknowledge the outstanding faculty at College of the Atlantic, especially my undergraduate advisors Chris W.

Petersen, Ken Cline, and John Anderson who have been very influential in shaping the way I approach science. I would also like to thank my MSc advisor Jes S e Pedersen for giving me valuable preparation for setting out into the world of research.

Finally, I am extremely grateful to my wonderful family and friends for all their support and for giving me the time and space to focus when my work demanded it. A special thanks to my Sami for the endless encouragement and understanding and for the invaluable help in keeping me on track, keeping my spirits up and keeping the right perspective during these last busy months.

Thank you all!

Berkeley, June 2012

A handwritten signature in blue ink, appearing to read 'Nina Overgaard Therkildsen', with a stylized, flowing script.

Nina Overgaard Therkildsen

Thesis summary

Accelerated rates of climate change and other growing anthropogenic pressure challenge the persistence of many plant and animal populations. Faced with drastically altered environmental conditions, natural populations may either go extinct locally or respond by: 1) dispersing to areas with more favorable conditions; 2) modifying their trait expression to better fit with new local conditions through phenotypic plasticity; or 3) adapt genetically in response to selection. A better understanding of the relative importance of these different coping mechanisms and the interactions between them in different settings will be crucial for making reliable predictions about the future distribution of biodiversity.

The present thesis uses genomic analysis of historical and contemporary DNA samples to retrospectively assess how populations of Atlantic cod, *Gadus morhua*, have responded to environmental and human-induced changes over the past century. Capitalizing on unique collections of archived samples and recently developed genomic resources, we study temporal and spatial variation at both microsatellite loci and up to 1047 gene-associated single nucleotide polymorphisms (SNPs) over a period of 80 years. The extensive sampling in time and the – for a non-model species – high genomic coverage provide unprecedented resolution for disentangling effects of drift, migration and selection. This elucidation generates novel insights about how cod previously have responded microevolutionarily to changed conditions. Focusing on two different cod population complexes, our overarching objectives have been to assess 1) whether levels of genetic diversity, population structure and distribution patterns have remained stable over time despite large demographic changes, 2) if we could detect molecular signatures of selection over decadal time scales and if so, how widespread such signatures would be, 3) if recent changes in selection pressures have been gradual or abrupt and what factors may have driven them, 4) how temporal and spatial variation in selection pressure have interacted, and 5) if signatures of recent selection are parallel between adjacent populations and across different geographic regions.

The thesis is divided into six chapters that report on different efforts to address these objectives. It opens with a general introduction and synopsis that lays out the context for the research, summarizes the main findings and discusses perspectives for future research (Chapter 1). Chapter 2 presents a baseline study demonstrating temporal stability at microsatellite loci over an 80-year period in a Canadian cod population. This result sets the stage for two SNP-based spatiotemporal population genomics studies that search for signatures of recent selection and examine the stability of population structure in Canadian (Chapter 3) and Greenlandic (Chapter 4) cod populations. The final two chapters elaborate on methodological developments that were implemented to reach the primary research goals: Chapter 5 evaluates the quality of SNP genotyping in our historical samples and demonstrates high reproducibility of our presented data while Chapter 6 proposes a new statistical approach to detecting loci under selection based on temporal variation in allele frequencies.

Our results provide important insights about the recent dynamics at both study locations. In Canada, the population structure appears to have remained intact over the study period, whereas in Greenland the temporal analyses reveal large changes in the spatial distribution and mixing of different populations. Against this backdrop of contrasting neutral evolutionary patterns, we observe widespread signatures of selection in both systems, reflected in substantial locus-specific divergence in space and time. This suggests ongoing adaptation in response to temporally and spatially varying selection pressures, and nonparallel patterns between adjacent areas indicate that populations may react differently to environmental variation. Both dispersal and adaptation hence appear to be important responses to environmental change in cod populations, depending on local conditions. These findings have important implications for our understanding of local adaptation and evolutionary potential in high gene flow organisms and underscore the need to carefully consider all dimensions of biocomplexity for evolutionarily sustainable management of biodiversity and natural resources.

Dansk resumé

Klimaforandringer og andre former for menneskeskabt påvirkning truer den fremtidige eksistens af mange dyre- og plantearter. Under drastisk ændrede miljøforhold kan vilde bestande enten uddø lokalt eller reagere ved 1) at bevæge sig til områder med mere gunstige forhold; 2) at modificere deres fysiologiske egenskaber gennem fænotypisk plasticitet; eller 3) at tilpasse sig genetisk i respons til selektion. En bedre forståelse af den relative vigtighed af disse overlevelsesmekanismer – og samspillet mellem dem – vil være afgørende for at kunne forudsige den fremtidige fordeling af biodiversitet.

Denne afhandling anvender genomisk analyse af historiske og nutidige DNA-prøver til retrospektivt at undersøge hvordan torskbestande (*Gadus morhua*) har reageret på naturlige og menneskeskabte ændringer i deres miljø over det seneste århundrede. Ved hjælp af unikke samlinger af arkiverede prøver og nyudviklede genomiske ressourcer studerer vi den tidlige og rumlige variation for både mikrosatellit loci og op til 1047 single nucleotide polymorphisms (SNPs) over en 80-årig periode. Den omfattende prøvetagning og den, for en ikke-model art, høje genomiske dækning giver hidtil uset opløsning til at udrede de historiske effekter af drift, migration og selektion. Denne udredning skaber ny indsigt i hvordan torsk tidligere har reageret mikroevo-lutionært på ændrede forhold. Med fokus på to forskellige bestandskomplekser har vores overordnede formål været at undersøge 1) om mængden af genetiske variation og populationsstrukturen er forblevet stabile over tid på trods af store demografiske ændringer, 2) om vi kan detektere molekulære signaturer fra selektion over meget korte tidsskalaer (årtier), og hvis det er tilfældet, hvor udbredte sådanne mønstre er, 3) om nylige ændringer i selektionstryk er sket gradvist eller pludseligt, og hvilke faktorer der har skabt ændringerne, 4) hvordan tidlig og rumlig variation i selektionstryk har spillet sammen, og 5) om vi ser parallelle genetiske ændringer i nabo-populationer og på tværs af geografiske regioner.

Afhandlingen er delt ind i 6 kapitler, som beskriver forskellige aspekter af vores indsats for at besvare disse spørgsmål. Der indledes med en generel introduktion og synopsis, som opridser konteksten for forskningsprojektet, opsummerer hovedkonklusionerne og diskuterer perspektiver for fremtidig forskning (Kapitel 1). Kapitel 2 præsenterer et baseline studie, som demonstrerer tidlig stabilitet for mikrosatellit loci over en 80-årig periode i en canadisk torskbestand. Dette resultat sætter scenen for to SNP-baserede spatio-temporale population genomics studier, hvor vi undersøger om der er tegn på nylig selektion og om populationsstrukturen er stabil i canadiske (Kapitel 3) og grønlandske (Kapitel 4) torskbestande. De to sidste kapitler beskriver metode-udvikling, som vi foretog for at nå projektets hovedmål: Kapitel 5 evaluerer kvaliteten af SNP genotypning i vores historiske prøver og demonstrerer en høj grad af reproducerbarhed af vores præsenterede data, mens Kapitel 6 foreslår en ny statistisk metode til at identificere loci under selektion ud fra tidlig variation i allelfrekvenser.

Vores resultater giver vigtig ny indsigt i torskbestandenes dynamik ved begge studielokaliteter. I Canada så populationsstrukturen ud til at være forblevet intakt over perioden, mens den tidlige analyse i Grønland viste store ændringer i geografisk fordeling og opblanding af forskellige bestande. Op imod denne baggrund af modsatrettede neutrale evolutionære mønstre, observerede vi udbredte tegn på selektion i begge systemer, afspejlet i stærke locus-specifikke afvigelser fra de generelle mønstre i både tid og rum. Dette tyder på igangværende tilpasning i respons til tidligt og rumligt varierende selektionstryk og ulige mønstre i forskellige områder indikerer at bestandene reagerer forskelligt på miljøvariation. Det ser derfor ud til at både ændringer i udbredelsesområdet og genetiske tilpasninger er vigtige reaktioner på miljøforandringer i torskbestande afhængigt af de lokale forhold. Disse erkendelser har vigtige konsekvenser for vores forståelse af lokale tilpasning i organismer med højt gen flow og understreger behovet for at indregne alle dimensioner af biokompleksitet for at opnå en evolutionær bæredygtig forvaltning af biodiversitet og naturressourcer.

CHAPTER 1

General introduction and summary of findings

Nina Overgaard Therkildsen

1. INTRODUCTION

1.1 Background

Growing anthropogenic pressure now challenges the persistence of many plant and animal populations (e.g. Hughes *et al.* 1997; Brook *et al.* 2008; Maclean & Wilson 2011). A diverse array of direct impacts such as pollution, habitat degradation, introduction of invasive species, and overexploitation add to accelerated rates of climate change to drastically alter the environmental conditions for species around us. Phenotypic plasticity—the ability of a single genotype to exhibit variable phenotypes in different environments—enables organisms to cope with many of these changes through adjusted trait expression, so that they can remain in their habitat without genetic adaptation. This mechanism has been crucial for mediating responses to rapid environmental shifts in many natural populations (Charmantier *et al.* 2008; Gienapp *et al.* 2008; Hendry *et al.* 2008), but physiological and evolutionary constraints impose limits on its capacity (Dewitt *et al.* 1998; Auld *et al.* 2010).

In response to environmental change - human-caused or otherwise - that exceeds the tolerance limits achievable through current phenotypic plasticity, populations may either go extinct locally, disperse to areas with more favorable conditions or adapt genetically in response to selection—all processes of potential evolutionary consequence. A better understanding of the relative importance of these processes and the interactions between them in different settings will be essential for making reliable predictions about the future distribution of biodiversity.

1.2 Focus of the thesis

To this end, the present thesis applies retrospective monitoring of genetic variation in natural populations of Atlantic cod (*Gadus morhua*) to examine how they have responded to human-induced and natural changes to their environment over the past century. The overarching aim has been to elucidate the roles that adaptation and distributional shifts have played in shaping these

responses and this way shed light on the extent, as well as the temporal and spatial patterns, of recent microevolution in the species.

This chapter introduces the context for the research project and discusses its main findings in relation to current knowledge in the field. I will first briefly elaborate on the different evolutionary responses to changing environments and outline how genetic methods in general, and temporal genetic analyses in particular, are useful for studying these processes in natural populations (Section 2). I will then consider how general characteristics of marine fish affect potential evolutionary responses (Section 3) and introduce the study species (Section 4). With the stage set, Section 5 will present the specific objectives for the thesis research and Section 6 will describe the applied methodology. I will follow with a summary of my findings in the different studies and my overall conclusions (Section 7), and finally discuss the implications of these findings as well as perspectives for future research (Section 8).

2. WHY USE MOLECULAR GENETIC METHODS TO STUDY EVOLUTIONARY CHANGE?

This section will briefly discuss how population responses to changed environmental conditions can have evolutionary consequences and outline why DNA-based methods are powerful tools to study these processes.

2.1 Demographic changes

Human activities that either create adverse conditions for growth and reproduction, impose direct harvesting, or affect the carrying capacity of habitats can strongly reduce the sizes of natural populations or alter their structure. Because genetic drift scales inversely with effective population size, such changes can result in loss of genetic diversity, and quantification of molecular variation at polymorphic sites across the genome is the most direct way to study this. There are indeed numerous documented examples of human-induced erosions of genetic diversity, for example due to fishing or hunting (e.g. Ryman *et al.* 1995; Allendorf *et al.* 2008) or population fragmentation caused by habitat destruction (e.g. Dixo *et al.* 2009; Struebig *et al.* 2011).

Loss of genetic variation can adversely affect population viability, both by reducing individual fitness in the short term and by impairing the ability to evolve in the future (Allendorf *et al.* 2008). Clearly, loss of functionally important variation can be detrimental in both the short and long term, but the implications of reduced diversity at neutral markers—that are typically used in population genetic studies—are less clear. A central question is whether the level of variation at neutral loci is similar to the variation at functionally important loci (e.g. quantitative trait loci), so that neutral loci reflect variation that is important for fitness and evolutionary potential.

A considerable research effort has been devoted to exploring this issue, and although many studies have failed to detect a correlation between heterozygosity at neutral markers and individual fitness, this correlation has been clear in other contexts, particularly in small populations (Frankham *et al.* 2002; Szulkin *et al.* 2010). Similarly, studies comparing molecular variation at neutral loci with the quantitative genetic variation at ecologically important traits have varied immensely in their conclusions and overall suggest only a weak correlation (Reed & Frankham 2001). This pattern of weak correlation between the signal from neutral markers and adaptive variation also seems to emerge from studies that compare levels of differentiation between populations (Merilä & Crnokrak 2001; McKay & Latta 2002; Holderegger *et al.* 2006).

These findings could suggest that neutral variation is a poor proxy for population viability and evolutionary potential. However, selection is always context-specific and loci that are neutral or nearly neutral now may become subject to selection under different environmental conditions. This means that neutral variation could potentially become adaptive variation in the future, so apparently neutral variation could represent a crucial part of a population's evolutionary legacy. Neutral markers therefore remain useful monitoring tools as indicators of genome-wide levels of variation, but they can increasingly be supplemented with markers directly associated with functional genes for more comprehensive insights about patterns of diversity (Allendorf *et al.* 2010).

2.2 Adaptation

Altered selection pressures that act on traits with a heritable basis can theoretically cause adaptive changes over very short time. The literature contains many examples of rapid adaptation in wild populations over contemporary time scales, especially in response to human impacts (e.g. Kinnison & Hendry 2001; Palumbi 2001; Stockwell *et al.* 2003; Smith & Bernatchez 2008). However, it is still unclear how widespread such short-term adaptive changes are, and under what conditions they occur at rates fast enough to track environmental and human-induced changes (Hendry *et al.* 2008; Hoffmann & Sgrò 2011).

Progress in elucidating these important questions has been hampered by the notorious difficulty in demonstrating a genetic basis for apparent local adaptations in natural populations (Gienapp *et al.* 2008; Hoffmann & Willi 2008). There are multiple strategies for disentangling the effects of phenotypic plasticity from genetic differences underlying observed trait variation (recently reviewed by Hoffmann & Sgrò 2011; Hansen *et al.* 2012). Most approaches involve either laboratory experiments such as common garden/reciprocal transplant setups or quantitative genetic techniques that require knowledge of family relationships—both undertakings that can be logistically prohibitive with large, long-lived and highly abundant organisms. For such systems, molecular genetic methods often offer more accessible opportunities for detecting signatures of adaptive divergence. Because selection affects the pattern and distribution of genetic variation both within and between populations, its signatures can often be detected in samples collected directly from the wild (Nielsen 2005; Storz 2005). The recent advances in sequencing and genotyping technologies make these molecular approaches even more powerful because increased genome coverage result in higher chances of identifying regions under selection (Luikart *et al.* 2003; Allendorf *et al.* 2010).

2.3 Shifts in distribution and migration patterns

Although distributional shifts *per se* do not necessarily have evolutionary impacts, they are often associated with altered patterns of gene flow between populations, which can have profound genetic consequences. A number of human activities such as overexploitation or habitat destruction can potentially affect species distribution patterns, but climate change has by far received the most attention as a driving force of distributional shifts. Overwhelming evidence makes it clear that global temperature increases have already caused substantial shifts in the range of many species, both terrestrial and marine (e.g. Root *et al.* 2003; Parmesan 2006; Chen *et al.* 2011). Studies that document these patterns and those that predict future impacts (e.g. Deutsch *et al.* 2008; Cheung *et al.* 2009) provide important insights. However, they typically focus on the species level, ignoring that species are made up of populations which each may harbor unique adaptations to specific local environments and therefore will react differently in response to altered conditions (Hilborn *et al.* 2003; Schindler *et al.* 2010; Kelly *et al.* 2011).

In most cases, it is unclear to what extent climate-induced species distribution shifts simply reflect the sum of different populations moving to new areas as they track the changing location of their environmental “niche”. Alternatively, species-level shifts may result from extinction of certain populations—and therefore loss of a unique portion of the species’ evolutionary legacy—coupled with local growth and spatial expansion in previously marginal populations. Understanding population diversity, and the extent to which populations are adapted to different conditions is therefore critical for making accurate predictions about the future distribution of biodiversity. As already mentioned, genetic methods are useful in this context because they can identify signatures of adaptive divergence. They are also, however, powerful tools for inferring basic population structure (e.g. Pritchard *et al.* 2000; Corander *et al.* 2003; Jombart *et al.* 2010), for tracing individuals to their population of origin (Manel *et al.* 2005; Nielsen *et al.* 2012), and for estimating contemporary and historical migration rates (Paetkau *et al.* 2004; Hey & Nielsen 2004; Yamamichi & Innan 2012).

2.4 The power of genetic monitoring

A primary reason why molecular tools are useful for studying evolutionary forces in the wild is that patterns of genetic diversity typically integrate effects accumulated over millennia. This means that cumulative impacts of even weak patterns can be detectable, but it makes it challenging to distinguish signatures of historical events predating colonization of current habitats from ongoing evolutionary changes. Hence, snapshot observations of the current distribution of genetic variation often tell us little about how stable these patterns are over time or how quickly they may change in response to human activities.

Temporally spaced DNA samples offer a unique opportunity for studying genetic change directly. This approach has been termed genetic monitoring and can either rely on pre-planned recurrent sampling or on archived or resurrectable samples, as well as data from previous studies, to obtain retrospective insights (Schwartz *et al.* 2007). By comparing the genetic composition of a population before and after a change in environmental conditions, it is possible to track changes in allele frequencies ‘in real time’ for direct assessment of genetic impacts. Previously, studies using presumably neutral markers have offered important insights about neutral processes including effective population sizes, loss of diversity, and stability of population structure and migration rates (see reviews by Leonard (2008), Wandeler (2007), and Nielsen and Hansen (2008)). Also, studies targeting specific candidate genes expected to be under selection have provided insights about the temporal dynamics of adaptive variation (e.g. Umina *et al.* 2005; Jensen *et al.* 2008; Marsden *et al.* 2012).

With the advances in molecular techniques, efforts to study temporal adaptive genetic variation are no longer limited to genes *a priori* expected to be under selection. Instead, it is now possible to screen large panels of genetic markers and apply genome scan approaches (see Section 6.4) to identify loci that are likely affected by selection. Genome scans are often used in comparisons of samples collected across space (Luikart *et al.* 2003; Storz 2005; Stinchcombe & Hoekstra 2007), but has only rarely been utilized to identify signatures of selection and ongoing adaptation over time in wild populations (notable examples are Hansen *et al.* 2010; Poulsen *et al.* 2011; Bourret *et al.* 2011; Orsini *et al.* 2012). This paucity of temporal applications probably results from technical constraints and limited sample availability. Yet, where such challenges can be overcome, simultaneous assessment of adaptive genetic variation at both the temporal and spatial scales over which different evolutionary forces are acting, offers extraordinary prospects for gaining more comprehensive insights into the potential for rapid adaptation.

3. EVOLUTIONARY RESPONSES TO CHANGE IN MARINE FISH

This section will outline some basic characteristics of marine fish and discuss the implications of these characteristics for studying evolutionary responses to change.

3.1 Why study marine fish?

Marine fish are useful models for studying responses to global change for a number of reasons. First, they are typically widely distributed across a range of ecological gradients, so it is possible to comparatively study impacts in different settings (Nielsen *et al.* 2009a). Being ectotherms, they are also more intimately linked with their environment than most terrestrial organisms, so may respond more readily to change (Cossins & Crawford 2005). At the same time, many species have been subjected to substantial and quantifiable human pressure in the form of intensive fisheries. This allows for important comparisons of how natural and human-induced influences interact in shaping evolutionary trajectories.

The perhaps most essential attributes in relation to genetic monitoring, however, are the extensive collections of archived samples that are available for many fish species. Owing to commercial interest, scales and otoliths have been collected systematically from many major fish stocks over the past century (initially for age and growth rate determination). Where these samples have been retained, they probably make up the most comprehensive collections of archived material from natural populations of any taxa, providing unparalleled opportunities for retrospective genetic monitoring (Nielsen & Hansen 2008).

3.2 General characteristics

While fish represent an extremely diverse group of species with a variety of life histories, behaviors, and physiologies, they can be grouped into general types. This thesis focuses on what has been called “classical” marine fish, which are characterized by large population sizes, high fecundity, high dispersal ability of adults, and pelagic eggs and larvae (Nielsen & Kenchington 2001; Nielsen *et al.* 2009a). Many commercially harvested species fit in this category and typically exhibit weak, but often highly significant, levels of population structure (Waples 1998; Hauser & Carvalho 2008). The following sections will discuss how these general characteristics are expected to influence potential evolutionary responses to change.

3.2.1 Large population sizes

With population sizes that typically exceed millions of individuals, it may be expected that selection has a greater impact on evolutionary trajectories in marine fish than it does in species with smaller populations, in which selection to a greater extent is counteracted by drift—another reason why fish are useful models to study effects of global change. The impact of evolutionary forces, however, depends on the effective population size (N_e), not the census size (N), and a large body of research indicates that there can be extremely large discrepancy between these parameters in marine organisms.

Recent studies have suggested that effective sizes of marine fish populations typically range from a few hundreds to a few thousands even though their census sizes are often two to five orders or magnitude higher (reviewed by Hauser & Carvalho 2008; Palstra & Ruzzante 2008). Such extremely small $N_e:N$ ratios are expected to result primarily from high variance in reproductive

success, as has been termed sweepstakes recruitment (Hedgecock 1994; Hedgecock & Pudovkin 2011). It has been demonstrated theoretically that this effect can substantially decrease $N_e:N$ ratios (Waples 2002; Hedrick 2005). However, the extent of skew in reproductive success required to explain observed patterns appears in some cases inconsistent with ecological observations (Flowers *et al.* 2002; Poulsen *et al.* 2006). The very high polymorphism observed at microsatellite loci in marine fish is also hard to reconcile with very small N_e 's, unless the N_e 's were reduced very recently or mutation rates are higher than currently expected (Poulsen *et al.* 2006; Palstra & Ruzzante 2008). Furthermore, the overall similarity of reported estimates across wildly different species and habitats (see Table 1 in Hauser & Carvalho 2008) could also reflect general limitations of genetic methods to estimate contemporary N_e when it is so large that sampling error may exceed signatures of drift (see Waples 1989; 1998).

If reported estimates indeed reflect biological reality, marine fish may be susceptible to loss of genetic diversity in face of demographic perturbations, because such N_e 's are within the range typically considered at risk (Frankham *et al.* 2002). Two pioneering genetic monitoring studies reported loss of both heterozygosity and allelic variation at microsatellite loci over decades of intense exploitation in marine fish populations (Hauser *et al.* 2002; Hutchinson *et al.* 2003). These studies have received a lot of attention in the literature as illustrations of conservation concern for marine fish. However, almost all subsequent temporal genetic studies based on a range of fish species including herring, eel, tuna, ray, cod, and sole have revealed stable levels of genetic diversity despite fisheries-induced population collapses (e.g. Hoarau *et al.* 2005; Poulsen *et al.* 2006; Nielsen *et al.* 2007; Han *et al.* 2008; Chevolot *et al.* 2008; Therkildsen *et al.* 2010; Riccioni *et al.* 2010; Larsson *et al.* 2010; Cuveliers *et al.* 2011; Jakobsdottir *et al.* 2011). These findings indicate that although human-induced diversity loss may be possible in marine fish, it is far from a universal consequence of fishery collapse and the debate about realistic magnitudes of N_e in marine fish populations remains open. Certainly, Chapters 2, 3, and 4 of this thesis demonstrate that it is always important to consider alternative causes such as genotyping error, selection and distributional shifts to explain observed temporal allele frequency changes.

3.2.2 Potential for high gene flow

With high dispersal ability of both pelagic and adult life stages and a habitat characterized by few obvious barriers, marine fish have traditionally been expected to exhibit high levels of gene flow. Such gene flow could limit adaptation to local conditions and temporal changes in these, because the diversifying effect of localized selection pressures would be swamped by the homogenizing effect of gene flow. For this reason, it was previously expected that local adaptation would be rare or absent in marine fish (Hauser & Carvalho 2008).

However, an accumulating number of recent studies based either on genomic signatures of selection on specific loci (e.g. Hemmer-Hansen *et al.* 2007; Nielsen *et al.* 2009b; Bradbury *et al.* 2010) or on common garden experiments (Marcil *et al.* 2006; e.g. Conover *et al.* 2006; Grabowski *et al.* 2009) have provided strong evidence for adaptive divergence in marine fish, even over surprisingly small spatial scales across which neutral genetic markers have typically have revealed very limited levels of population structure (Hutchings *et al.* 2007; Olsen *et al.* 2008; Poulsen *et al.* 2011). Strong local selection pressures (Olsen & Moland 2011) and low realized levels of gene flow due to homing behaviors (Robichaud & Rose 2004; Svedäng & Righton 2007) and larval retention (Cowen *et al.* 2006; Knutsen *et al.* 2007; Bradbury *et al.* 2008) have been suggested as mechanisms that enable adaptation in these systems. Regardless of the particular interplay of factors, the evidence supporting adaptive divergence across a range of spatial scales strongly indicate that temporal variation in selection pressures also may leave genomic signatures in marine fish.

The potential for high gene flow must, however, be carefully considered as an alternative to selection in explaining temporal shifts in allele frequencies. An unavoidable—but often underappreciated—challenge in genetic monitoring is to clarify whether you have sampled a single population over time or if you have sampled different populations that moved in and out of the study area (Hansen *et al.* 2012). In principle, a genome scan approach (see Section 6.4), should be able to disentangle effects of migration and selection because it is based on the premise that migration should have relatively homogenous effects across the entire genome, whereas selection should only affect specific gene regions (Lewontin & Krakauer 1973; Luikart *et al.* 2003). However, when the genetic differentiation to potential migrant source populations is very low across neutral loci but higher at loci under selection, this separation of evolutionary forces can become challenging. We address this issue with simulations in Chapter 3, whereas in Chapter 4 we observed higher baseline levels of differentiation allowing for better disentanglement of the effects.

3.2.3 Fisheries-induced selection

Direct exploitation has probably been the main human impact on fish populations over time. Fisheries have been called large-scale uncontrolled experiments in evolutionary selection (e.g. Rijnsdorp 1993, Stokes and Law 2000) because fish harvest often constitutes a dominant source of mortality for the affected populations (up to four times the natural mortality (Jørgensen *et al.* 2007)) and is almost always non-random with respect to fish phenotypes (e.g. typically fish of a certain size or behavior are targeted). When there is a heritable component to the phenotypic variation among fish, such strong selective mortality can cause adaptive change. Life history theory and empirical evidence suggest that fisheries this way can induce evolutionary changes in many demographically and ecologically important traits (see Law 2000, Heino and Godø 2002, Munch *et al.* 2005). Some of these changes can have highly adverse effects on the productivity and resilience of fish stocks, thus potentially jeopardizing future yields and sustainability (Stokes & Law 2000; Heino & Godø 2002; Conover & Munch 2002).

Despite the clear theoretical foundations and a substantial research investment, it has, however, been difficult to determine whether exploited stocks are indeed undergoing fisheries-induced evolution, and if so over what time scales. The resulting uncertainty coupled with the potentially severe consequences has sparked a high-profiled debate about whether fisheries management should account for the evolutionary dimension of exploitation, and the issue remains unresolved (e.g. Jørgensen *et al.* 2007; Hilborn & Minto-Vera 2008; Browman *et al.* 2008; Kuparinen & Merilä 2008).

Given the difficulty of studying the genetic basis of trait variation in the wild, most efforts to inform this debate have been based on indirect methods, primarily empirical analyses of decadal trends in observed trait variation in exploited stocks (reviewed in Jørgensen *et al.* 2007) and modeling studies (e.g. Ernande *et al.* 2004; Dunlop *et al.* 2009). While the combined evidence generated through these approaches strongly indicate evolutionary effects, methodological shortcomings have prevented unequivocal proof that observed changes are truly genetic: Since models necessarily are simplifications of reality, it is difficult to evaluate their biological realism without empirical testing, and studies of directly observable trait changes in nature suffer from difficulties in disentangling the effects of environmental and genetic influences on trait expression (Kuparinen & Merilä 2007; Browman *et al.* 2008). A robust method for filtering away the effects of environmental variation involves bringing animals into controlled settings so that observed trait variation should reflect genetic differences only. Such experimental simulations have delivered an important proof-of-concept, demonstrating empirically that fisheries selection can cause substantial genetic changes in the exploited populations (Conover & Munch 2002; Reznick & Ghalambor 2005; Conover & Baumann 2009). However, due to logistic constraints, these studies have been based

on short-lived model organisms in highly simplified set-ups, and it is unclear whether the findings can be scaled up to the complex dynamics of commercial fish populations in the wild.

Retrospective genetic monitoring of these wild populations provides one of the most promising avenues for elucidating the genetic basis of apparent fisheries-induced evolution. With the increasing ability to track functionally important genetic variation, progress towards this goal is already being made (Chapters 3 and 4). However, a complete understanding of fitness effects underlying observed genetic changes and interactions between fishing and other drivers of selection will probably only emerge through substantial dedicated research efforts that integrate the powers of different approaches (Conover & Baumann 2009). A particularly valuable approach in this context will be to combine spatiotemporal genetic studies in the wild with genetic studies under common garden conditions.

4. THE STUDY ORGANISM: ATLANTIC COD (*Gadus morhua*)

This section will describe the basic biology of the study organism and provide an overview of the human pressure it has been exposed to through fisheries. Following the general species-level information, I will briefly introduce the specific populations studied in the thesis.

4.1 Why Atlantic cod?

We based our studies on Atlantic cod because it has historically been one of most important commercial fish species in the North Atlantic and owing to this commercial interest, as well as its pivotal ecological role in many marine ecosystems, has received enormous research attention. It is thus among the most well-studied marine fish, providing valuable background knowledge on its ecology, reproduction and population structure. Extensive collections of archived cod otoliths also exist in many North Atlantic fisheries research institutions, and with a published full genome sequence (Star *et al.* 2011) and hundreds of curated EST-derived SNPs placed on a linkage map (Hubert *et al.* 2010; Bowman *et al.* 2011), it probably has more genomic resources available than any other non-model fish species. This combination of sample availability and genomic tools enables extensive exploration of functional genetic variation in both time and space.

4.2 Basic biology

As a prime example of a classical marine fish, the Atlantic cod is characterized by mobile adults, high fecundity, external fertilization, and pelagic eggs and larvae. It can live for up to 25 years, and exhibiting indeterminate growth like other teleost fish, can reach lengths of 2 meters (Cohen *et al.* 1990) and weights of almost 100 kg (Frimodt 1995). However, very few individuals now attain this age and size and populations are generally made up of considerably smaller fish. Spawning occurs in multiple batches over a period of 3-6 weeks and a female typically produce between 300,000 and millions of eggs in a breeding season (Kjesbu *et al.* 1996; Chambers & Waiwood 1996). Fecundity scales with body size so larger females produce more eggs. There is considerable variation in the life history parameters between different populations and the age at maturity ranges from 2-3 years in some areas and up to 8 years in others (McIntyre & Hutchings 2003).

Some cod populations undertake annual migrations of 100s of kilometers while in other populations, adults remain sedentary all year (Robichaud & Rose 2004). However, during the

pelagic egg and larval stage, dispersal is governed by local oceanographic conditions. Eggs hatch after about 60 degree-days and the larval phase lasts about 750 degree-days (Cohen *et al.* 1990), i.e. the duration of the pelagic stage in water of 8°C is about 100 days. Mortality rates are high during the early life stages and the survival rate from birth until age 3 years has been estimated to be around 10^{-6} , so one in a million (Hutchings 1999). Adult cod live demersally (it is called a “groundfish”) and inhabit coastal areas and offshore continental shelves throughout the North Atlantic. In the eastern part, it ranges from the North Sea northward to the Barents Sea off Norway and northern Russia and across to Iceland and Greenland. In the Northwest Atlantic, it ranges from Cape Hatteras in the south to Baffin Island in the North (Mieszkowska *et al.* 2009).

4.3 Fishing impacts

Human exploitation of cod can be traced back at least 1000 years (Barrett *et al.* 2008). Its widespread abundance throughout the North Atlantic has supported some of the world’s largest and economically most important fisheries and fishing pressure has been intense on all populations throughout its range (Mieszkowska *et al.* 2009). Global landings of this species peaked already in the late 1960s when the total annual landings were almost 3 million tons (FAO 2000). Since then landings have declined steadily and are now at about one fourth of the maximum level (FAO 2000). This drop in landings results from collapses in several important fisheries and decreased abundance in others. The most renown of these collapses has occurred in the historically very large cod fisheries off eastern Canada. These stocks have suffered dramatic declines of up to 99.9% compared to historic levels and were completely closed to fishing in the early 1990s (Hutchings & Reynolds 2004). Also in the Northeast Atlantic, many stocks have declined over the past decades, and population collapse has been imminent in many areas (Mieszkowska *et al.* 2009). While several factors may have contributed to these widespread declines, overexploitation has been implicated as the main cause in many cases (Myers *et al.* 1997; Pauly *et al.* 2002).

As a consequence of the sharp declines in abundance, researchers have debated the extinction vulnerability of cod, and several populations have been added to governmental and intergovernmental lists of threatened or endangered species (COSEWIC 2003; Dulvy *et al.* 2005; Reynolds *et al.* 2005). The conservation concern arises in part because recovery from low abundance level may be extremely slow. Many collapsed fish stocks show no or little change in abundance after more than 15 years following the collapse (Hutchings 2000; 2001). Many different factors may contribute to the lack of recovery, including depensation (the Allee effect), changes in species compositions, and habitat modifications (see Hutchings & Reynolds 2004). However, it has also been hypothesized that genetic changes may play a role (Hutchings 2005; Enberg *et al.* 2009).

A large proportion of studies documenting fisheries-induced evolution in life history traits have been conducted on cod populations. These have documented substantial reductions in either growth rate or timing of reproduction over the past decades in cod throughout its range (e.g. Jørgensen *et al.* 2007; Swain *et al.* 2007; Pardoe *et al.* 2009). As discussed above, statistical treatment of observed trait variation in the wild do not provide entirely conclusive evidence for the genetic basis of changes. Questions have also been raised about whether distributional shifts and population replacement, rather than adaptive evolution, can explain the observed changes (Andersen & Brander 2009). However, the combined evidence of parallel trends in multiple studies illustrates the pervasiveness of a common pattern. Getting a better understanding of whether the changes indeed reflect ongoing adaptation—and therefore potentially much more slowly reversible than purely phenotypic changes (Law 2000; Salinas *et al.* in press)—will be important for clarifying their role in stock recovery and persistence. It will also be important with more detailed

investigation into the nature of the selection pressure imposed by fishing and how this selection interacts with other evolutionary forces to shape population responses.

4.4 Population structure

Decades of population genetics research have revealed extensive population structure in Atlantic cod over both small and large spatial scales throughout its range (reviewed in Hauser & Carvalho 2008). In addition to a deep split between European and North American cod populations (O Leary *et al.* 2007; Bigg *et al.* 2008; Pampoulie *et al.* 2008), cod are subdivided into many smaller units on each side of the Atlantic, and genetic differentiation has been observed down to the level of individual fjords (Jorde *et al.* 2007) or between depth strata within the same area (Pampoulie *et al.* 2006). There is still little documentation for the long-term stability of such extreme microgeographic differentiation, but many studies report short-term stability of observed population structure over a few years, and in some cases stability over decades has been confirmed (e.g. Ruzzante *et al.* 2001; Poulsen *et al.* 2006; Nielsen *et al.* 2007). This stability supports the biological realism of the observed structure, although the level of differentiation is generally very low, often with $F_{ST} < 0.01$ on regional scales (Hauser & Carvalho 2008; Knutsen *et al.* 2011). This low differentiation may reflect high levels of gene flow between populations, but could also indicate recent divergence and large N_e 's. Tagging studies and larval dispersal surveys in many cases suggest that ongoing exchange among populations is limited (e.g. Robichaud & Rose 2004; Svedäng & Righton 2007; Bradbury *et al.* 2008), and recent findings of very strong divergence at specific loci (e.g. Nielsen *et al.* 2009b; Bradbury *et al.* 2010; Nielsen *et al.* 2012) may support this notion. Even though adaptation is possible in face of strong gene flow if differences local selection pressures are strong enough to overcome the homogenizing effects of migration (Lenormand 2002; Sambatti & Rice 2006), such signatures of selection may also reflect a high degree of current isolation that is not yet visible at neutral markers because large N_e 's have limited accumulation of drift since divergence.

4.5 The study populations

In this thesis, we study two very different cod population complexes. Chapters 2 and 3 focus on a central part of the cod distribution range around the Gulf of St. Lawrence in Canada where cod probably have been present for many millennia, potentially pre-dating the last glacial maximum (LGM; Bigg *et al.* 2008). All the populations in this region have suffered population collapses due to overfishing and have all failed to recover, in part because of major habitat changes (Hutchings & Reynolds 2004). These populations thus represent a system that has been highly dominated by direct human impact for many decades, but more recently also have been affected by a number of significant environmental changes.

Chapter 4, in contrast, focuses on the northern range edge for cod in waters around Greenland, which currently represents marginal habitat, reflected in a non-constant occurrence of cod (Buch *et al.* 1994; Rätz & Lloret 2005). This region appears to have been colonized by cod after the LGM (Bigg *et al.* 2008) and variation in local conditions have, at least over the past few centuries, spurred episodic outbursts of cod abundance interchanged with periods of virtual absence from some areas (Hansen 1949; Buch *et al.* 1994). This highly dynamic system has probably been dominated by environmental influences, although human overexploitation has also had important demographic impacts at times (Buch *et al.* 1994; Rätz & Lloret 2005).

5. RESEARCH OBJECTIVES

In the two described populations complexes, we wanted to address the following overarching questions:

- Are levels of genetic diversity, population structure and distribution patterns stable over time despite large demographic changes?
- Can we observe molecular signatures of selection over decadal time scales?
- If so, what is the genomic pattern (i.e. which and how many locations in the genome are affected, does selection appear to have been gradual or abrupt, and can we identify potential drivers of the selection)?
- What is the interaction between selection in space and time?
- Are signatures of recent selection parallel between adjacent populations and across different geographic regions?

6. METHODS

This section will introduce the main methodology we applied to meet the research objectives. I will describe the rationale for choosing the particular methods and briefly outline their power and limitations.

6.1 Genetic markers

We applied two different types of genetic markers: microsatellites (Chapter 2) and single nucleotide polymorphisms (SNPs; Chapters 3-6). Microsatellites are short tandem repeats of 1-6 nucleotides and alleles are distinguished by varying number of repeat units (i.e. total length). The fragmented nature of historical DNA limits microsatellite analysis in genetic monitoring to loci with maximum allele sizes below ~250 bp, but many microsatellites fulfill this criterion and constitute valuable resources (Wandeler *et al.* 2007; Nielsen & Hansen 2008).

Although it is well known that some microsatellites occur within genes or are part of upstream regulatory elements, they are generally expected to be embedded in non-coding sequence and of no functional effect, therefore reflecting effects of neutral evolutionary forces and demographic history (Beebee & Rowe 2008). However, if a microsatellite locus is linked to a proximate functional locus, it may be subject to hitchhiking selection, which can substantially bias analyses based on assumptions of neutrality (Luikart *et al.* 2003; Nielsen *et al.* 2006). We applied microsatellites for a baseline assessment of temporal trends in genetic variation (Chapter 2) because their mutation rates typically are much higher than other nuclear loci, and thus they are more polymorphic (Li *et al.* 2002). Such loci with many rare alleles provide maximal power for detecting recent loss of genetic variation because allelic diversity is a much more sensitive indicator of population bottlenecks than heterozygosity (Cornuet & Luikart 1996; Allendorf *et al.* 2008).

SNPs are single base substitutions and represent the simplest and most abundant form of genetic variation, widely distributed throughout all genomes. For analyses of degraded DNA, a technical advantage of SNPs is that their genotyping typically requires only a short (<100 bp) flanking sequence for each locus, so they can be analyzed even in highly fragmented DNA (provided the quality is adequate). Recent developments also allow high throughput simultaneous analysis of

1000s of SNPs either through genotyping-by-sequencing (Elshire *et al.* 2011; Davey *et al.* 2011) or chip/array-based genotyping platforms (Edenberg & Liu 2009).

These technologies provide unprecedented resolution for studying genomic patterns and allow separation of genome-wide from locus-specific variation, which is a powerful approach for detecting effects of selection (Luikart *et al.* 2003; Allendorf *et al.* 2010). SNPs are common both in functional and non-coding parts of the genome, so panels can be selected based on study objectives. Following our baseline assessment that indicated no temporal change at microsatellite loci in the Canadian population (Chapter 2), we wanted to search for signatures of selection. With this goal in mind, we based the remainder of the studies on a highly targeted panel consisting exclusively of SNPs developed from expressed sequences (Moen *et al.* 2008; Hubert *et al.* 2010; Bowman *et al.* 2011). With the limited (although much increased compared to previous studies) genome coverage currently achievable for population studies of non-model organisms, this allowed us to focus on functional genetic variation, which is most likely to have been affected by selection. Using SNPs located in genic regions hence maximized our chances of detecting signatures of selection and, as an added advantage, will facilitate future efforts to understand the phenotypic effect of interesting polymorphisms.

Our non-random selection of loci would introduce ascertainment bias for certain types of analysis (e.g. Rosenblum & Novembre 2007; Albrechtsen *et al.* 2010). However, since our study is based on observed shifts in allele frequencies, not the allele frequency spectrum itself, our SNP selection should not cause biases in the temporal analysis. Nevertheless, as a result of the targeted sampling of loci, our results cannot easily be extrapolated to the rest of the genome and hence do not necessarily provide robust estimates of the proportion of the genome that is affected by short-term selection.

6.2 Estimation of N_e

To better understand the evolutionary trajectories of the study populations and to quantify genome-wide levels of genetic variation between sampling points, we estimated N_e 's based on observed temporal variation in allele frequencies. This is the most commonly applied approach for estimating contemporary N_e (Palstra & Ruzzante 2008; Luikart *et al.* 2010) and is based on the principle that if effects of migration, selection and mutation can be assumed negligible, observed genetic changes over a given number of generations will reflect genetic drift, the magnitude of which is inversely proportional to the effective population size (Waples 1989; Wang 2005).

A number of estimators have been proposed based on this principle (reviewed by Wang 2005; Leberg 2005; Luikart *et al.* 2010). Traditional moment-based methods (Krimbas & Tsakas 1971; Nei & Tajima 1981; Waples 1989) remain popular because of their simple computations and their direct interpretations in relation to classical population genetics theory. In recent years, a number of more computationally intensive methods have been developed based on e.g. maximum likelihood (Anderson *et al.* 2000; Wang 2001), Bayesian coalescent inference (Berthier *et al.* 2002), or approximate Bayesian computation (Tallmon *et al.* 2004). Because these newer methods tend to use more information in the data, they are expected to be superior to moment-based methods both regarding accuracy and precision. Several comparative simulation studies have confirmed that this indeed is often the case (Wang 2001; Berthier *et al.* 2002; Tallmon *et al.* 2004), although not in all situations (Jorde & Ryman 2007).

In Chapter 2, we compare the results from several different N_e estimators, including a completely different type of method that is based on the amount of linkage disequilibrium (LD) observed within single samples rather than temporal changes between samples (Waples & Do 2008). We found that many methods could not distinguish the N_e from infinity, indicating that it is so large that there

was no observable drift signal in the data after accounting sampling error. It is well-known that genetic methods have limited power to distinguish large from very large N_e 's (Nei & Tajima 1981; Luikart *et al.* 2010), but even when an accurate quantification is not possible, these methods can be useful for confirming that the N_e of a population is not small. Our comparative study found relatively consistent results from multiple methods that are based on different models with varying assumptions and different mathematical approaches (Chapter 2), so we proceeded to only apply a single method (either likelihood or moment-based) for the remaining chapters.

6.3 Discriminant analysis of principle components (DAPC)

Computation of F-statistics or genetic distances from *a priori* defined groupings of individuals probably remains the most widely applied approach for describing genetic population structure. It can often be useful, however, to also apply clustering methods that decompose a sample of individuals into genetically distinct groups without *a priori* characterization of these groups. In recent years, Bayesian clustering methods such as Structure (Pritchard *et al.* 2000; Falush *et al.* 2003) or BAPS (Corander *et al.* 2003) have been among the most popular for this type of analysis. Based on minimizing the Hardy Weinberg and linkage disequilibrium that would arise if individuals from different randomly mating populations are mixed, these methods infer the most likely number of populations represented in a sample according to explicit population genetics models.

Such Bayesian clustering methods have been immensely important for improving our understanding of population structure in a wide range of species, but unfortunately, their power to detect and describe structure decreases with the degree of differentiation between groups, and they provide little useful information when differentiation is very low (e.g. Latch *et al.* 2006; Waples & Gaggiotti 2006). As corollary, we also found that the weak differentiation between samples in our studies here (mean pairwise $F_{st} < 0.017$, Chapters 3 and 4) generally did not allow meaningful or conclusive results from clustering individuals into populations based on Structure analysis.

In contrast, we found that a recently developed method, discriminant analysis of principle components (DAPC; Jombart *et al.* 2010) had greater power to extract useful information about clusters in our dataset. This multivariate method identifies groups of similar individuals through K-means clustering and then describes the relationship among these groups by constructing synthetic variables that maximize variance between and minimize variance within groups. One advantage of DAPC is that it does not rely on assumptions of particular population genetics models or a particular type of substructure. Further, in a comparative evaluation on simulated data, DAPC consistently showed similar or better performance than Structure for inferring the correct number of populations represented in a sample, and appeared clearly superior when the underlying simulation scenario was complex (Jombart *et al.* 2010).

In spite of the superior performance across the parameter space tested, DAPC may, like other clustering methods, sometimes infer artifacts of discrete groups in populations where genetic diversity is continuously distributed (Jombart *et al.* 2010). However, the distribution of membership probabilities for each individual to the identified clusters is indicative of how distinct clusters are, and scatter plots of the synthetic variables allow for a graphical assessment of how the genetic variability in the data is organized. The method thus has several features that are useful for interpreting the results, and although more extensive evaluation is required to fully understand its powers and limitations, DAPC seems like a very promising tool. It may also generally prove more powerful than alternative methods when differentiation is low, as indicated by our empirical experience (Chapter 4). It should be kept in mind, however, that the ability to pick up vague structure is always associated with a risk of confusing sampling noise or data errors with true biological signals (Waples 1998; Hedrick 1999). Therefore, results should always be interpreted

with due consideration of possible confounding factors, and where possible, temporal replication can considerably strengthen confidence in the results (Waples 1998).

6.4 Genome scans

A central goal of this thesis was to search for signatures of very recent selection in the studied cod populations. Selection can affect the pattern and distribution of genetic variation both within and between populations and species, and a number of statistical tests have been developed to identify these effects (see reviews by Nielsen 2001; 2005). Some of the most popular tests (e.g. Tajima's D or MacDonald-Kreitman) are based on signals that have accumulated within single populations over many, potentially thousands of, generations. Therefore, these methods are not spatially explicit and can typically not resolve the temporal dimension of variation in selection pressures. Because we here were interested in divergence between populations and in directional selection occurring over the study period, we instead applied genome scan methods (Storz 2005; Beaumont 2005) that compare differences in observed allele frequencies among population samples.

The basic premise underlying genome scan methods (also called outlier tests) is that neutral evolutionary forces like migration and drift are expected to exert relatively uniform effects across the entire genome, whereas selection is expected to act only on specific loci and closely linked sites (Cavalli-Sforza 1966; Lewontin & Krakauer 1973). Therefore, genomic regions that show divergent patterns of differentiation are likely affected by selection, either as the direct target or through hitch-hiking effects (Lewontin & Krakauer 1973; Luikart *et al.* 2003; Storz 2005).

As was recognized in the initial formalized test based on this principle, it can be applied to characterize variation both in space between different populations and in time within the same population (Lewontin & Krakauer 1973; 1975). However, almost all subsequent statistical refinements of the method have focused on the between-population scenario (e.g. Beaumont & Nichols 1996; Beaumont & Balding 2004; Foll & Gaggiotti 2008; Excoffier *et al.* 2009). Such spatial methods, which assume that samples are collected at a single time point from geographically separated populations exchanging migrants, have occasionally been applied to temporal data based on serial sampling of a single population (e.g. Bourret *et al.* 2011; Poulsen *et al.* 2011; Orsini *et al.* 2012). While this type of application may yield some insights, the results can be hard to interpret because the clear violation of the inherent population genetic model leaves uncertainties about the reliability of p-values or posterior probabilities for a locus being a significant outlier (see Hansen *et al.* 2012). To ameliorate this problem, we adapted a commonly applied outlier detection method to explicitly evaluate outlier status in relation to a temporal scenario (Chapter 6) and applied this in combination with conventional spatial tests for integrated spatiotemporal analysis (Chapters 3 and 4).

Although genome scan methods are increasingly popular in studies of natural populations, the underlying approach has been severely criticized in a number of recent papers (reviewed by Barrett & Hoekstra 2011; Li *et al.* 2011). The criticisms mainly concern whether the models applied to generate neutral expectations adequately account for effects of demographic history (Thornton & Jensen 2007; Hermisson 2009), whether it makes sense to assume that neutral evolutionary forces have equal baseline effects across the genome (Buerkle *et al.* 2011), whether selection indeed only affects localized regions of the genome or if it has much more pervasive effects than previously appreciated (Hahn 2008), and whether outlier status implicate adaptive significance (Barrett & Hoekstra 2011). While all these concerns certainly have some validity, they mostly relate to the certainty of conclusions that can be drawn about locus-specific effects or to uncharacterized downstream fitness effects of particular outliers. Genome scans remain useful tools to identify the genomic regions that are most likely to have been affected by selection and thereby generate

important candidate loci for follow-up studies that can further clarify specific selection patterns and functional roles.

6.5 Landscape genomics

In addition to genome scans, we used a landscape genomics approach to test for associations between environmental parameters and temporal and spatial variation in allele frequencies. The underlying principle here is that strong correlations can suggest selection driven by particular environmental variables (or correlated factors) and hence help generate hypotheses about causative agents for observed patterns (Joost *et al.* 2007; Manel *et al.* 2010a; Coop *et al.* 2010). To account for the allele frequency patterns that arise due to factors such as differences in sample sizes and shared history among some populations, we primarily applied a recently developed Bayesian method that incorporates a null model based on covariance at putatively neutral loci (Coop *et al.* 2010).

Generally, landscape genomics methods have been developed to compare spatial patterns of allele frequency variation to some average measure of environmental conditions at the sampling sites (e.g. Joost *et al.* 2007; Manel *et al.* 2010b). This way, observed allele frequencies likely reflect accumulated responses to long-term exposure to particular environmental agents. Nevertheless, the principle is also applicable to tests for temporal correlations between allele frequency variations and environmental conditions at different sampling time points (Hansen *et al.* 2012). These correlations may be considerably more difficult to detect than spatial correlations because of uncertainties about cumulative effects and lag time in the responses. However, an assessment of broad patterns can be a very useful starting point for more detailed investigations to identify putative drivers of selection.

7. SUMMARY OF STUDIES AND FINDINGS

Below, I will briefly outline the content and main findings from each thesis chapter and discuss how the combined conclusions contribute to our understanding of recent microevolution in high gene flow species. The thesis opens with a baseline study on temporal genetic variation at neutral markers (Chapter 2) to set the stage for two retrospective population genomics studies that identify signatures of recent selection and examine the stability of population structures in the two very different cod population complexes (Chapters 3 and 4). As supporting material, two final chapters (5 and 6) report on methodological developments that were required to achieve the research objectives of the primary studies, and which will be useful for future research.

Chapter 2: Large effective population size and temporal genetic stability in Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence

This paper is based on work I started for my M.Sc. thesis but have substantially revised (including additional data analysis) during my Ph.D. studies. It is included here because it provides an important baseline for the subsequent studies. Based on extensively validated genetic data from historical and contemporary samples from the Gulf of St Lawrence cod population, we here found complete stability of allele frequencies at nine polymorphic microsatellite loci over 80 years, spanning a period from before the commercial fishery intensified to present time when the population is at historically low abundance. Over the period, we did not observe any loss of

heterozygosity or allelic diversity. As a consequence of the temporal stability, the majority of applied estimation methods could not distinguish the N_e from infinity. The lower 95% confidence limit on estimates was generally >500, suggesting that the effective population size is likely to be considerably larger.

These findings of genetic continuity, maintained levels of standing genetic variation and a large N_e , despite severe reductions in census size, were very promising for the prospects of detecting signatures of selection, because they indicated that drift and migration had not been important drivers of microevolution in this population over the study period. Accordingly, the temporal stability at neutral markers represents an ideal baseline for testing the hypothesis of recent selection, because any substantial locus-specific departures from temporal stability would likely reflect signatures of selection. Hence, this study set the stage for our more comprehensive SNP analysis of the system.

Chapter 3: Microevolution in time and space: SNP analysis of historical DNA reveals dynamic signatures of selection in Atlantic cod

This study was designed to follow up on the findings from Chapter 2 of long-term temporal stability at neutral loci in the southern Gulf of St. Lawrence cod, by now looking for genetic signatures of selection over the 80-year period. To obtain a better temporal resolution, we supplemented our previously analyzed samples with additional time points from the intervening period. Since the Gulf of St. Lawrence is a semi-enclosed basin that appears to contain a reproductively isolated population, we did not expect that population replacement could impact our results, but to control for this, and to assess interactions between temporal and spatial variation in selection pressures, we also included temporally spaced samples from three adjacent populations. Through genome scans based on an initial screening of >1000 SNPs in the temporally extreme samples and 160 SNPs in a follow-up panel, we identified 77 loci that showed highly elevated levels of differentiation in either time, space, or both. Temporal allele frequency shifts at certain loci correlated with local temperature variation or with fisheries-induced life history changes. Surprisingly, however, largely non-overlapping sets of loci were temporal outliers in the different populations and outliers identified in the 1928-2008 comparison showed almost complete stability from 1960-2008. The apparent contrasting micro-evolutionary trajectories among populations resulted in sequential shifts in which loci were spatial outliers, with no locus maintaining elevated differentiation throughout the study period.

To evaluate whether gene flow could have driven these outlier patterns, we conducted a series of simulations to assess how much migration would be needed to generate the observed allele frequency changes. We found that migration rates of >0.2 would be required, but such high rates are highly inconsistent with ecological data and observations of temporally stable (though weak) spatial structure at neutral loci. This suggests that disintegrated population structures or shifting migration patterns alone cannot explain the observed allele frequency changes at outlier loci, indicating that they are likely driven by highly dynamic temporally and spatially varying selection.

Chapter 4: Spatiotemporal SNP analysis reveals pronounced biocomplexity at the northern range margin of Atlantic cod *Gadus morhua*

In contrast to Chapter 3 that focused on populations from the central part of the species' distribution range where cod has continuously been present at least since the 1500s, this chapter focused on the northern edge of cod distribution, which currently is marginal habitat, reflected in a non-constant occurrence of cod (Buch *et al.* 1994). With the forecasted warming, however, this

region is predicted to become an important area for the species (Drinkwater 2005). Hence this system represents an exciting opportunity to study ongoing climate-change induced colonization of northern habitats. Related to earlier environmental fluctuations, cod has episodically exhibited dramatic outbursts of abundance in the waters around Greenland over the past centuries (Hansen 1949; Buch *et al.* 1994). It has, however, been unclear to what extent such rapid increases in abundance have arisen through recurrent colonization by populations from elsewhere (Iceland) or if they result from sudden growth in resident populations that have maintained a stable distribution through periods of rarity. Also, the degree of reproductive isolation among previously hypothesized population components was unknown, as were potential adaptive differences among these components.

In this manuscript, we, as in Chapter 3, used a spatiotemporal population genomics approach to examine the temporal stability of population structure and identify signatures of divergent selection over a period of 78 years spanning major demographic changes. In this system, however, we *a priori* expected a more dynamic population structure and focused particularly on elucidating this aspect along with assessing potential adaptive differences. By genotyping >900 SNPs in almost 850 individuals collected through extensive sampling from spawning grounds both contemporarily and during a historical period of maximum abundance, we identified four genetically distinct groups that over time exhibited varying spatial distributions with considerable overlap and mixing. At some spawning grounds, the genetic composition remained stable over decades, whereas complete population replacement was evident at others. We observed highly elevated differentiation in certain genomic regions, which is consistent with adaptive divergence between the groups. This indicates that they may harbor diverging adaptations to the Arctic environment and therefore will respond differently to environmental variation. Significantly increased temporal changes at a subset of loci also suggest that adaptation may be ongoing and that the populations possess potential for rapid response to altered selection pressures.

Overall, this study illustrates a highly dynamic system where different population components exhibit divergent distribution patterns, potentially due to differential response to environmental changes. Yet, the continued presence of all populations even over decades of virtual absence of cod in certain regions indicates considerable resilience in this system of biocomplexity and suggests that historical fluctuations have been caused by combinations of local population growth and external influx.

Chapter 5: Evaluation of a high-throughput SNP genotyping platform for analysis of degraded DNA from historical fish samples

This technical note describes the performance of the Illumina GoldenGate SNP genotyping assay for our historical DNA samples analyzed in Chapters 3 and 4. This assay is based on hybridization of allele-specific fluorescently labeled primers to the template DNA and can genotype up to 3072 SNPs simultaneously, providing a powerful medium-throughput platform for population genomics in non-model organisms. Developed for high-quality DNA, we had not previously seen this platform used for historical samples, so we did not *a priori* know if it would be applicable for our work. However, the genotyping is based on short (60 bp) oligonucleotide probes, suggesting that it could be robust to DNA fragmentation typical of degraded historical samples.

Fortunately, we found remarkably high genotyping quality for the majority of our samples. Our analysis identified a number of factors that correlated with reproducibility and call rate, providing valuable information for the planning of future studies. We also demonstrated that after quality filtering which maximized data retention while limiting potential inaccuracies, the genotyping error rate was always <3% (much less for certain samples) and generally >90% of samples were

successfully genotyped for each SNP. These results strongly support the reliability of data presented in Chapters 3 and 4.

Chapter 6: Ftemp: A method to detect genomic signatures of selection from temporal sampling

This chapter describes a methodological adjustment that was needed to identify signatures of selection from temporal genetic data. As described in Section 6.4, practically all modern genome scan methods are developed for spatial comparisons of allele frequencies between populations and hence are based on models that do not necessarily provide a good characterization of variation over time within a single population. In this paper, we propose a modification of the commonly applied genome scan method *fdist* (Beaumont & Nichols 1996). Our approach compares the observed temporal differentiation at individual loci to a neutral expected distribution generated through simulations of drift within a single population. We demonstrate that the model generally is robust to uncertainty in parameter input values and that it responds as expected to variations in sampling configuration. Application to example datasets shows that it identifies a larger number of temporal outliers than methods designed to evaluate spatial patterns, but that the strongest outliers are consistently identified by all methods. The main strength of our proposed approach is that it provides an intuitive and simple framework that generates readily interpretable statistical thresholds for analysis of temporal data.

The method is currently only implemented in personal R-scripts, but with additional collaborators (Tiago Antao and Samitha Samaranayake), we are exploring the potential for developing it into a user-friendly java-application for public release.

Overall conclusions and implications

With the powerful combination of large panels of gene-linked SNPs and sampling that spans several populations over multiple decades, Chapters 3 and 4 represent—to my knowledge—the most extensive temporal genetic studies on natural populations of any non-model species conducted to date. For this reason, they provide unprecedented insight into how selection operates and interacts with other forces over—in evolutionary terms—short time scales in presumably high gene flow organisms.

The spatiotemporal approach was common to both studies, but the higher temporal resolution in the Canada study versus the emphasis on spatial resolution in Greenland makes the findings highly complementary. In both cases, however, the sampling over time was essential for understanding recent patterns in population structure and spatial distributions and for demonstrating that these can either show complete stability or dramatic changes over periods with large demographic fluctuations, depending on local conditions. In the Canadian populations, the level of genetic differentiation between populations was very low, except at a few outlier loci that showed increased divergence at different time points. This pattern made it difficult to draw strong conclusions about the temporal stability of population structure from the observed allele frequencies at non-outlier loci alone. However, the historical genetic data was useful to parameterize simulations that, in combination with ecological data, suggested that migration or population replacement could not explain the very large changes in allele frequencies we observed at certain loci, and that the population structure has remained stable despite the low differentiation.

In the Greenlandic populations, the level of differentiation was also too low for successful application of several standard methods for inferring population structure. However, with a

multivariate approach, we were able to assign the majority of individuals to one of four identified populations with high statistical support. Through this assignment of individuals collected in both historical and contemporary time, we could directly observe how the genetic composition of cod in specific locations changed over time. We saw that in some areas, almost all sampled individuals originated from the same population and that this pattern was consistent between sampling years. In other areas, population samples appeared to contain individuals originating from different populations. Sometimes the constitution of these mixtures seemed stable over time while they varied between sampling years in other locations. In the most extreme cases, both the historical and the contemporary samples from a location were relatively “pure”, but assigned to different populations, suggesting complete population replacement. Monitoring the genetic origin of fish at different points in space and time therefore provided an important tool for tracking changes in the spatial distribution and mixing patterns of the different overlapping populations. Understanding this dynamic population structure was important both for elucidating the neutral microevolution in the system and for providing a baseline against which to detect signatures of selection.

Both of our main studies suggested that signatures of selection were widespread, causing divergence in both space and time. In Canada (Chapter 3), the outlier loci on average showed more variation among sampling times within populations than they did in spatial comparisons among populations. This indicates that at least over the time scale considered, temporal variation in selection pressures had a greater impact than spatially varying selection in shaping allele frequencies in this system. This contrasts with the Greenland study (Chapter 4) where the average spatial variation at outlier loci exceeded the temporal variation. Another interesting difference is that in Canada, most outliers showed elevated divergence in both space and time, whereas there was almost no overlap between spatial and temporal outlier loci in Greenland.

These differences may be caused by a number of factors. Variation in the power to detect outliers may play a role due to the inherent incongruence in sampling designs. However, the much larger geographical scale considered in Greenland could also explain why spatial variation in selection pressures would be larger in this system. Additional studies will be needed to further elucidate interactions between temporal and spatial scales of selection in marine fish. The few previous studies that have screened temporal trends in either single genes or smaller panels of gene-linked SNPs have provided mixed results with some reporting complete stability (Nielsen *et al.* 2007; 2009b; Poulsen *et al.* 2011) and others notable changes at particular loci (Árnason *et al.* 2009; Jakobsdottir *et al.* 2011; Poulsen *et al.* 2011).

The relatively large allele frequency shifts we observed here within populations suggest that temporal variations in selection pressures are pronounced. This may have to do with the short time scale we consider, as a number of studies have demonstrated that in nature, selection typically shows large fluctuations in both direction and strength, and that short term variation often exceeds long term averages (Kinnison & Hendry 2001; Hairston *et al.* 2005; Siepielski *et al.* 2011). In any case, our results suggest that selection has strongly affected the Canadian cod populations over the study period, potentially reflecting ongoing adaptation to changing conditions. Considering how dominant fisheries have been in shaping mortality patterns here, it is likely that this human impact has at least partially contributed to the selection pressures, although changing environmental conditions probably also have been important. We draw a similar conclusion about the Greenlandic cod populations, but our results also make it clear that dynamic and contrasting dispersal patterns—probably driven by a number of environmental influences—also have played an important role in this system.

While slightly different SNP panels were applied, a number of loci showed strong outlier patterns in both studies, making these particularly interesting candidates for follow-up studies to clarify their functional roles and potential fitness effect (see below). Among the common outliers was a group of SNPs in strong linkage disequilibrium (LD) that spanned > 20 cM on the linkage map. Notably,

however, each study also showed signs of strong selection on an additional group of strong LD SNPs that did not show outlier behavior in the alternate study. These patterns indicate that both common and contrasting regions of the genome are involved in adaptive responses to different environmental variables in both space and time.

Although the phenotypic and fitness effects of observed outlier loci remain unknown, the findings here add an important perspective to recent studies presenting evidence for local adaptation in marine fish either through elevated divergence at specific loci (Nielsen *et al.* 2009b; Bradbury *et al.* 2010) or common garden experiments (Marcil *et al.* 2006; Grabowski *et al.* 2009; Harrauld *et al.* 2010). Where temporal stability has not been demonstrated, it can clearly not be assumed *a priori*. If environmental conditions are highly dynamic, local adaptation may also not necessarily imply static differences between populations, but can reflect ongoing changes.

The abundance of both temporal and spatial outlier loci suggest that selection have played an important role in shaping the recent microevolution in the studied populations and that cod can respond rapidly to changes in selection pressure. Future investigations will help reveal the extent to which these signatures translate to adaptive trait changes, but the dynamic patterns suggest marine fish populations are reacting to human-induced and natural modifications of their environment through a number of mechanisms.

8. FUTURE PERSPECTIVES

This thesis illustrates how technological developments continue to enable entirely novel insights about the distribution of biodiversity in nature and fundamentally expand the range of questions we can address in our research. Such technology-driven insights have certainly influenced molecular studies of vagile marine organisms. As mentioned above, these species were previously expected to be genetically homogeneous and relatively panmictic throughout their range, but the advent of highly polymorphic markers completely transformed our understanding of how widespread population structure is (Hauser & Carvalho 2008). Similarly, the increasing ability to screen large panels of genetic markers is beginning to reveal how pronounced and abundant signatures of divergent selection appear to be (Nielsen *et al.* 2009b; Bradbury *et al.* 2010; Pespeni *et al.* 2012). Now, as the next wave, we can extend population genomics to the temporal dimension and start to discover how selection pressures vary in time and interact with other forces to shape evolutionary trajectories over short temporal scales.

Yet, we are only at the dawn of this new research approach, and genetic monitoring of both neutral and functional genetic variation has enormous potential that has still to be fully unleashed. There are many exciting avenues for future research based both on application of tools that are already available, on taking advantage of rapid improvements in sequencing methods, and on coupling population genomics with alternative disciplines. In the following, I will briefly outline how I envision that follow-up investigations could further build on our existing results.

8.1 Higher temporal resolution

The purpose of this thesis research was to gain a synoptic overview of overarching temporal patterns in the two study populations and generally to assess the potential for identifying signatures of ongoing selection through retrospective monitoring over decadal time scales. The promising results will provide the basis for more detailed follow-up studies that focus on a finer temporal resolution. For example, more frequent sampling coupled with distinction between age

classes will likely provide deeper insights into how selection operates and fluctuates over time and will allow more strongly supported hypotheses about what factors are driving it. It will also allow more fine-grained analysis of how environmental factors have affected the spatial distribution of different subpopulations and interactions among these.

In addition, archaeological samples, e.g. fish bones from historical human settlements, can extend studies even further back in time, potentially centuries or millennia (Barrett *et al.* 2008). The DNA quality of such samples may prohibit broad screening of hundreds of markers, but it may be possible to genotype individual candidate loci for longer-term assessments of distribution patterns and responses to selection (e.g. Svensson *et al.* 2007; Watson & Lockwood 2009).

8.2 Better genome coverage

Although we here study temporal variation at a much greater number of markers than has been available in the past for non-model organisms, we are clearly only capturing a fraction of the genomic signatures of selection. The decreasing cost and increasing throughput of sequencing and genotyping technologies will enable much denser marker coverage in the future (Allendorf *et al.* 2010; Davey *et al.* 2011), which will be important for more comprehensive insights into the genome-wide signatures of selection.

However, although entire genomes can now be sequenced even from ancient specimen (Rasmussen *et al.* 2010), population studies will still for some time face a strong trade-off between the density of genomic coverage and the number of samples analyzed, and technological limitations for degraded historical samples remain. If these limitations are taken into account, various genotyping-by-sequencing methods (Elshire *et al.* 2011; Davey *et al.* 2011) may prove the most efficient for future research although high throughput genotyping platforms (Edenberg & Liu 2009) also have advantages. Depending on specific project goals, it will be important to decide whether to target loci that are randomly distributed in the genome (e.g. developed from restriction enzyme-based methods (Davey *et al.* 2011)) or to focus efforts on genic regions (e.g. identified with RNA-Seq (Wang *et al.* 2009)). The advantage of the former approach is a more systematic survey of genome-wide patterns, whereas the latter allows better coverage in regions most likely to be affected by selection and thus easier association with functional impacts.

Before extending the genomic coverage to completely new regions, however, there is also much additional information to gain from surveying more closely the genomic neighborhoods of the candidate outlier loci identified here. Our results suggested that several outliers were located in extensive LD groups and more fine-grained investigation of these regions will be important for narrowing in on the specific targets of selection. The fragmented nature of the current cod genome assembly (Star *et al.* 2011) does not presently allow full overview of the sequence variation surrounding outlier SNPs, but coupled with the linkage map (Hubert *et al.* 2010) it provides a useful tool for future targeted re-sequencing of specific regions.

8.3 Understanding the adaptive significance

While the molecular signatures of recent selection are informative in themselves, a full understanding of their role in short-term adaptation to altered conditions requires that links between genotype, phenotype, fitness, and selective drivers be established (Stinchcombe & Hoekstra 2007; Nielsen 2009; Barrett & Hoekstra 2011). Generating this full line of evidence is a notoriously challenging feat that has only very rarely been achieved in natural populations (see Barrett & Hoekstra 2011).

Methods such as association mapping may help reveal genotype-phenotype correlations in the wild (Ellegren & Sheldon 2008; Slate *et al.* 2008), but it is often difficult to disentangle complex interactions, especially in large populations that cannot practically be pedigreed. Therefore progress towards a better understanding of the adaptive significance will to a large extent depend on controlled laboratory studies. Integration of molecular and quantitative genetics methods in such set-ups would help reveal the genetic basis underlying phenotypic traits (Stinchcombe & Hoekstra 2007; Naish & Hard 2008), but would be logistically challenging due to the long life span of cod and other commercial fish. Long-term collaborations with the aquaculture industry may prove fruitful in this respect because of the shared interest in understanding the genetic basis of key traits.

Once genotype-phenotype links are established, it will be important to examine how phenotypic variation translates into fitness effects in the wild and how these change over time. The extensive monitoring data accumulated through decades of stock assessment research on commercial fish stocks provide a valuable resource for this and for estimating selection differentials on specific traits (e.g. Swain *et al.* 2007; Kendall & Quinn 2012). Correlations between the temporal trends in such differentials or the raw genotypic and phenotypic variation and historical records of environmental variation or human pressure can provide compelling evidence for causative drivers of selection. However, multi-generational selection experiments provide the strongest evidence for how particular factors shape the fitness landscape and cause adaptive change (Conover & Baumann 2009). While such undertakings are almost impossible with Atlantic cod, studies of model organisms (e.g. Conover & Munch 2002; Reznick & Ghalambor 2005) may provide parallel insights and can identify candidate genes for use in retrospective monitoring of wild populations.

8.4 Exciting times ahead

The rapid advances in sequencing technology are currently revolutionizing population genetics and—especially in combination with inferences from other disciplines—provide unprecedented insights about adaptation and the distribution of genetic variation in natural populations. By extending these insights to the temporal dimension, genetic monitoring will undoubtedly play an important role in improving our understanding of how different microevolutionary processes play out and help clarify how populations will respond to rapid changes. The opportunities are only starting to unfold.

LITERATURE CITED

- Albrechtsen A, Nielsen FC, Nielsen R (2010) Ascertainment biases in SNP chips affect measures of population divergence. *Molecular Biology and Evolution*, **27**, 2534–2547.
- Allendorf FW, England PR, Luikart G, Ritchie PA, Ryman N (2008) Genetic effects of harvest on wild animal populations. *Trends in Ecology & Evolution*, **23**, 327–337.
- Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetics. *Nature Reviews Genetics*, **11**, 697–709.
- Andersen KH, Brander K (2009) Expected rate of fisheries-induced evolution is slow. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 11657–11660.
- Anderson EC, Williamson EG, Thompson EA (2000) Monte Carlo evaluation of the likelihood for N_e from temporally spaced samples. *Genetics*, **156**, 2109–2118.
- Auld JR, Agrawal AA, Relyea RA (2010) Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **277**, 503–511.
- Árnason E, Hernandez UB, Kristinsson K (2009) Intense habitat-specific fisheries-induced selection at the molecular *Pan* I locus predicts imminent collapse of a major cod fishery. *PLoS ONE*, **4**, e5529.
- Barrett J, Johnstone C, Harland J *et al.* (2008) Detecting the medieval cod trade: a new method and first results. *Journal*

- of *Archaeological Science*, **35**, 850–861.
- Barrett RDH, Hoekstra HE (2011) Molecular spandrels: tests of adaptation at the genetic level. *Nature Reviews Genetics*, **12**, 767–780.
- Beaumont MA (2005) Adaptation and speciation: what can F_{ST} tell us? *Trends in Ecology & Evolution*, **20**, 435–440.
- Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology*, **13**, 969–980.
- Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **263**, 1619–1626.
- Beebe T, Rowe G (2008) *An Introduction to Molecular Ecology*. Oxford University Press, USA.
- Berthier P, Beaumont MA, Cornuet J-M, Luikart G (2002) Likelihood-based estimation of the effective population size using temporal changes in allele frequencies: a genealogical approach. *Genetics*, **160**, 741–751.
- Bigg GR, Cunningham CW, Ottersen G *et al.* (2008) Ice-age survival of Atlantic cod: agreement between palaeoecology models and genetics. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **275**, 163–173.
- Bourret V, O'Reilly PT, Carr JW, Berg PR, Bernatchez L (2011) Temporal change in genetic integrity suggests loss of local adaptation in a wild Atlantic salmon (*Salmo salar*) population following introgression by farmed escapees. *Heredity*, **106**, 500–510.
- Bowman S, Hubert S, Higgins B *et al.* (2011) An integrated approach to gene discovery and marker development in Atlantic cod (*Gadus morhua*). *Marine Biotechnology*, **13**, 242–255.
- Bradbury IR, Hubert S, Higgins B *et al.* (2010) Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **277**, 3725–3734.
- Bradbury IR, Laurel BJ, Robichaud D *et al.* (2008) Discrete spatial dynamics in a marine broadcast spawner: Re-evaluating scales of connectivity and habitat associations in Atlantic cod (*Gadus morhua* L.) in coastal Newfoundland. *Fisheries Research*, **91**, 299–309.
- Brook B, Sodhi N, Bradshaw C (2008) Synergies among extinction drivers under global change. *Trends in Ecology & Evolution*, **23**, 453–460.
- Browman HI, Law R, Marshall CT (2008) The role of fisheries-induced evolution. *Science*, **320**, 47.
- Buch E, Horsted SA, Hovgård H (1994) Fluctuations in the occurrence of cod in Greenland waters and their possible causes. *ICES Mar. Sci. Symp.*, **198**, 158–174.
- Buerkle CA, Gompert Z, Parchman TL (2011) The $n = 1$ constraint in population genomics. *Molecular Ecology*, **20**, 1575–1581.
- Cavalli-Sforza L (1966) Population structure and human evolution. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **164**, 362–379.
- Chambers RC, Waiwood KG (1996) Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 1986–2003.
- Charmantier A, McCleery RH, Cole LR *et al.* (2008) Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, **320**, 800–803.
- Chen IC, Hill JK, Ohlemüller R, Roy DB, Thomas CD (2011) Rapid range shifts of species associated with high levels of climate warming. *Science*, **333**, 1024–1026.
- Cheung WWL, Lam VWY, Sarmiento JL *et al.* (2009) Projecting global marine biodiversity impacts under climate change scenarios. *Fish and Fisheries*, **10**, 235–251.
- Chevolot M, Ellis JR, Rijnsdorp AD, Stam WT, Olsen JL (2008) Temporal changes in allele frequencies but stable genetic diversity over the past 40 years in the Irish Sea population of thornback ray, *Raja clavata*. *Heredity*, **101**, 120–126.
- Cohen DM, Inada T, Iwamoto T, Scialabba N (1990) FAO species catalogue vol. 10. Gadiform fishes of the world (Order Gadiformes). An annotated and illustrated catalogue of cods, hakes, grenadiers and other gadiform fishes known to date. *FAO Fisheries Synopsis*, **10 (125)**, 442 p.
- Conover DO, Baumann H (2009) The role of experiments in understanding fishery-induced evolution. *Evolutionary Applications*, **2**, 276–290.
- Conover DO, Munch SB (2002) Sustaining fisheries yields over evolutionary time scales. *Science*, **297**, 94–96.
- Conover DO, Clarke LM, Munch SB, Wagner GN (2006) Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. *Journal of Fish Biology*, **69**, 21–47.
- Coop G, Witonsky D, Di Rienzo A, Pritchard JK (2010) Using environmental correlations to identify loci underlying local adaptation. *Genetics*, **185**, 1411–1423.
- Corander J, Waldmann P, Sillanpää MJ (2003) Bayesian analysis of genetic differentiation between populations. *Genetics*, **163**, 367–374.
- Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.
- COSEWIC (2003) *COSEWIC assessment and update status report on the Atlantic cod Gadus morhua in Canada*. Committee on the Status of Endangered Wildlife in Canada, Ottawa.
- Cossins AR, Crawford DL (2005) Fish as models for environmental genomics. *Nature Reviews Genetics*, **6**, 324–333.
- Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. *Science*, **311**, 522–527.
- Cuveliers EL, Volckaert F, Rijnsdorp A, Larmuseau M, Maes G (2011) Temporal genetic stability and high effective population size despite fisheries-induced life-history trait evolution in the North Sea sole. *Molecular Ecology*, **20**,

3555-3568.

- Davey JW, Hohenlohe PA, Etter PD *et al.* (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics*, **12**, 499–510.
- Deutsch CA, Tewksbury JJ, Huey RB *et al.* (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 6668–6672.
- Dewitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, **13**, 77–81.
- Dixo M, Metzger JP, Morgante JS, Zamudio KR (2009) Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. *Biological Conservation*, **142**, 1560–1569.
- Drinkwater KF (2005) The response of Atlantic cod (*Gadus morhua*) to future climate change. *ICES Journal of Marine Science*, **62**, 1327–1337.
- Dulvy NK, Jennings S, Goodwin NB, Grant A, Reynolds JD (2005) Comparison of threat and exploitation status in North-East Atlantic marine populations. *Journal of Applied Ecology*, **42**, 883–891.
- Edenberg HJ, Liu Y (2009) Laboratory methods for high-throughput genotyping. *Cold Spring Harbor Protocols*, **4**, 1–11.
- Ellegren H, Sheldon BC (2008) Genetic basis of fitness differences in natural populations. *Nature*, **452**, 169–175.
- Elshire RJ, Glaubitz JC, Sun Q *et al.* (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, **6**, e19379.
- Enberg K, Jørgensen C, Dunlop ES, Heino M, Dieckmann U (2009) Implications of fisheries-induced evolution for stock rebuilding and recovery. *Evolutionary Applications*, **2**, 394–414.
- Excoffier L, Hofer T, Foll M (2009) Detecting loci under selection in a hierarchically structured population. *Heredity*, **103**, 285–298.
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.
- FAO (2000) FISHSTAT Plus: Universal Software for fisheries statistics time series. Version 2.3 (FIDASU FAO Fisheries Department, Ed.).
- Flowers JM, Schroeter SC, Burton RS (2002) The recruitment sweepstakes has many winners: Genetic evidence from the sea urchin *Strongylocentrotus purpuratus*. *Evolution*, **56**, 1445–1453.
- Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, **180**, 977–993.
- Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, U.K.
- Frimodt C (1995) *Multilingual illustrated guide to the world's commercial coldwater fish*. Fishing News Books, Osney Mead, Oxford, England.
- Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J (2008) Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology*, **17**, 167–178.
- Grabowski TB, Young SP, Libungan LA, Steinarnsson A, Marteinsdottir G (2009) Evidence of phenotypic plasticity and local adaption in metabolic rates between components of the Icelandic cod (*Gadus morhua* L.) stock. *Environmental Biology of Fishes*, **86**, 361–370.
- Hahn MW (2008) Toward a selection theory of molecular evolution. *Evolution*, **62**, 255–265.
- Hairton NG Jr, Ellner SP, Geber MA, Yoshida T, Fox JA (2005) Rapid evolution and the convergence of ecological and evolutionary time. *Ecology Letters*, **8**, 1114–1127.
- Han YS, Sun YL, Liao YF *et al.* (2008) Temporal analysis of population genetic composition in the overexploited Japanese eel *Anguilla japonica*. *Marine Biology*, **155**, 613–621.
- Hansen MM, Meier K, Mensberg K-LD (2010) Identifying footprints of selection in stocked brown trout populations: a spatio-temporal approach. *Molecular Ecology*, **19**, 1787–1800.
- Hansen MM, Olivieri I, Waller DM, Nielsen EE, The GeM Working Group (2012) Monitoring adaptive genetic responses to environmental change. *Molecular Ecology*, **21**, 1311–1329.
- Hansen P (1949) Studies on the biology of the cod in Greenland waters. *Rapp. P.-V. Réun. Cons. Int. Explor. Mer*, **123**, 1–77.
- Harrald M, Wright PJ, Neat FC (2010) Substock variation in reproductive traits in North Sea cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 866–876.
- Hauser L, Carvalho GR (2008) Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. *Fish and Fisheries*, **9**, 333–362.
- Hauser L, Adcock GJ, Smith PJ, Ramirez JHB, Carvalho GR (2002) Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 11742–11747.
- Hedgecock D (1994) Does variance in reproductive success limit effective population sizes of marine organisms? In: *Genetics and Evolution of Aquatic Organisms* (ed Beaumont AR), pp. 122–134. Chapman and Hall, London.
- Hedgecock D, Pudovkin AI (2011) Sweepstakes reproductive success in highly fecund marine fish and shellfish: A review and commentary. *Bulletin of Marine Science*, **87**, 971–1002.
- Hedrick PW (1999) Perspective: Highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.
- Hedrick PW (2005) Large variance in reproductive success and the Ne/N ratio. *Evolution*, **59**, 1596–1599.
- Heino M, Godø OR (2002) Fisheries-induced selection pressures in the context of sustainable fisheries. *Bulletin of Marine Science*, **70**, 639–656.

- Hemmer-Hansen J, Nielsen EE, Frydenberg J, Loeschcke V (2007) Adaptive divergence in a high gene flow environment: Hsc70 variation in the European flounder (*Platichthys flesus* L.). *Heredity*, **99**, 592–600.
- Hendry AP, Farrugia TJ, Kinnison MT (2008) Human influences on rates of phenotypic change in wild animal populations. *Molecular Ecology*, **17**, 20–29.
- Hermisson J (2009) Who believes in whole-genome scans for selection? *Heredity*, **103**, 283–284.
- Hey J, Nielsen R (2004) Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics*, **167**, 747–760.
- Hilborn R, Quinn TP, Schindler DE, Rogers DE (2003) Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 6564–6568.
- Hoarau G, Boon E, Jongma DN *et al.* (2005) Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **272**, 497–503.
- Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. *Nature*, **470**, 479–485.
- Hoffmann AA, Willi Y (2008) Detecting genetic responses to environmental change. *Nature Reviews Genetics*, **9**, 421–432.
- Holderegger R, Kamm U, Gugerli F (2006) Adaptive vs. neutral genetic diversity: implications for landscape genetics. *Landscape Ecology*, **21**, 797–807.
- Hubert S, Higgins B, Borza T, Bowman S (2010) Development of a SNP resource and a genetic linkage map for Atlantic cod (*Gadus morhua*). *BMC genomics*, **11**, 191.
- Hughes JB, Daily GC, Ehrlich PR (1997) Population diversity: its extent and extinction. *Science*, **278**, 689–692.
- Hutchings JA (1999) Influence of growth and survival costs of reproduction on Atlantic cod, *Gadus morhua*, population growth rate. *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 1612–1623.
- Hutchings JA (2000) Collapse and recovery of marine fishes. *Nature*, **406**, 882–885.
- Hutchings JA (2001) Influence of population decline, fishing, and spawner variability on the recovery of marine fishes. *Journal of Fish Biology*, **59**, 306–322.
- Hutchings JA (2005) Life history consequences of overexploitation to population recovery in Northwest Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 824–832.
- Hutchings JA, Reynolds JD (2004) Marine fish population collapses: Consequences for recovery and extinction risk. *Bioscience*, **54**, 297–309.
- Hutchings JA, Swain DP, Rowe S *et al.* (2007) Genetic variation in life-history reaction norms in a marine fish. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **274**, 1693–1699.
- Hutchinson WF, Oosterhout CV, Rogers SI, Carvalho GR (2003) Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 2125–2132.
- Jakobsdóttir KB, Pardoe H, Magnússon Á *et al.* (2011) Historical changes in genotypic frequencies at the Pantophysin locus in Atlantic cod (*Gadus morhua*) in Icelandic waters: evidence of fisheries-induced selection? *Evolutionary Applications*, **4**, 562–573.
- Jensen LF, Hansen MM, Mensberg KLD, Loeschcke V (2008) Spatially and temporally fluctuating selection at non-MHC immune genes: evidence from TAP polymorphism in populations of brown trout (*Salmo trutta*, L.). *Heredity*, **100**, 79–91.
- Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, **11**, 94.
- Joost S, Bonin A, Bruford MW *et al.* (2007) A spatial analysis method (SAM) to detect candidate loci for selection: towards a landscape genomics approach to adaptation. *Molecular Ecology*, **16**, 3955–3969.
- Jorde PE, Ryman N (2007) Unbiased estimator for genetic drift and effective population size. *Genetics*, **177**, 927–935.
- Jorde PE, Knutsen H, Espeland SH, Stenseth NC (2007) Spatial scale of genetic structuring in coastal cod *Gadus morhua* and geographic extent of local populations. *Marine Ecology Progress Series*, **343**, 229–237.
- Jørgensen C, Enberg K, Dunlop ES *et al.* (2007) Managing evolving fish stocks. *Science*, **318**, 1247–1248.
- Kelly MW, Sanford E, Grosberg RK (2011) Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **279**, 349–356.
- Kendall NW, Quinn TP (2012) Quantifying and comparing size selectivity among Alaskan sockeye salmon fisheries. *Ecological Applications*, **22**, 804–816.
- Kinnison MT, Hendry AP (2001) The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica*, **112-113**, 145–164.
- Kjesbu OS, Solemdal P, Bratland P, Fonn M (1996) Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 610–620.
- Knutsen H, Olsen EM, Ciannelli L *et al.* (2007) Egg distribution, bottom topography and small-scale cod population structure in a coastal marine system. *Marine Ecology Progress Series*, **333**, 249–255.
- Knutsen H, Olsen EM, Jorde PE *et al.* (2011) Are low but statistically significant levels of genetic differentiation in marine fishes “biologically meaningful?” A case study of coastal Atlantic cod. *Molecular Ecology*, **20**, 768–783.
- Krimbas CB, Tsakas S (1971) Genetics of *Dacus oleae* V. Changes of esterase polymorphism in a natural population following insecticide control: selection or drift? *Evolution*, **25**, 454–460.
- Kuparinen A, Merilä J (2007) Detecting and managing fisheries-induced evolution. *Trends in Ecology & Evolution*, **22**, 652–659.

- Larsson LC, Laikre L, eacute CA, Dahlgren TG, Ryman N (2010) Temporally stable genetic structure of heavily exploited Atlantic herring (*Clupea harengus*) in Swedish waters. *Heredity*, **104**, 40–51.
- Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE Jr. (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, **7**, 295–302.
- Law R (2000) Fishing, selection, and phenotypic evolution. *ICES Journal of Marine Science*, **57**, 659–668.
- Leberg P (2005) Genetic approaches for estimating the effective size of populations. *Journal of Wildlife Management*, **69**, 1385–1399.
- Lenormand T (2002) Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*, **17**, 183–189.
- Leonard JA (2008) Ancient DNA applications for wildlife conservation. *Molecular Ecology*, **17**, 4186–4196.
- Lewontin RC, Krakauer J (1973) Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. *Genetics*, **74**, 175–195.
- Lewontin RC, Krakauer J (1975) Letters to the editors: Testing the heterogeneity of F values. *Genetics*, **80**, 397–398.
- Li J, Li H, Jakobsson M *et al.* (2011) Joint analysis of demography and selection in population genetics: where do we stand and where could we go? *Molecular Ecology*, **21**, 28–44.
- Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, **4**, 981–994.
- Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW (2010) Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conservation Genetics*, **11**, 355–373.
- Maclean IMD, Wilson RJ (2011) Recent ecological responses to climate change support predictions of high extinction risk. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 12337–12342.
- Manel S, Gaggiotti OE, Waples RS (2005) Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology & Evolution*, **20**, 136–142.
- Manel S, Joost S, Epperson BK *et al.* (2010a) Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. *Molecular Ecology*, **19**, 3760–3772.
- Manel S, Poncet BN, Legendre P, Gugerli F, Holderegger R (2010b) Common factors drive adaptive genetic variation at different spatial scales in *Arabidopsis alpina*. *Molecular Ecology*, **19**, 3824–3835.
- Marcil J, Swain DP, Hutchings JA (2006) Countergradient variation in body shape between two populations of Atlantic cod (*Gadus morhua*). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **273**, 217–223.
- Marsden CD, Woodroffe R, Mills MGL *et al.* (2012) Spatial and temporal patterns of neutral and adaptive genetic variation in the endangered African wild dog (*Lycaon pictus*). *Molecular Ecology*, **21**, 1379–1393.
- McIntyre TM, Hutchings JA (2003) Small-scale temporal and spatial variation in Atlantic cod (*Gadus morhua*) life history. *Canadian Journal of Fisheries and Aquatic Sciences*, **60**, 1111–1121.
- McKay JK, Latta RG (2002) Adaptive population divergence: markers, QTL and traits. *Trends in Ecology & Evolution*, **17**, 285–291.
- Merilä J, Crnokrak P (2001) Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology*, **14**, 892–903.
- Mieszkowska N, Genner MJ, Hawkins SJ, Sims DW (2009) Effects of climate change and commercial fishing on Atlantic Cod *Gadus morhua*. *Advances in marine biology*, **56**, 213–273.
- Moen T, Hayes B, Nilsen F *et al.* (2008) Identification and characterisation of novel SNP markers in Atlantic cod: Evidence for directional selection. *BMC Genetics*, **9**, 18.
- Myers R, Mertz G, Bridson J (1997) Spatial scales of interannual recruitment variations of marine, anadromous, and freshwater fish. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 1400–1407.
- Naish KA, Hard JJ (2008) Bridging the gap between the genotype and the phenotype: linking genetic variation, selection and adaptation in fishes. *Fish and Fisheries*, **9**, 396–422.
- Nei M, Tajima F (1981) Genetic drift and estimation of effective population size. *Genetics*, **98**, 625–640.
- Nielsen EE, Hansen MM (2008) Waking the dead: the value of population genetic analyses of historical samples. *Fish and Fisheries*, **9**, 450–461.
- Nielsen EE, Kenchington E (2001) A new approach to prioritizing marine fish and shellfish populations for conservation. *Fish and Fisheries*, **2**, 328–343.
- Nielsen EE, Cariani A, Aoidh EM *et al.* (2012) Gene-associated markers provide tools for tackling illegal fishing and false eco-certification. *Nature communications*, **3**, 851.
- Nielsen EE, Hansen MM, Meldrup D (2006) Evidence of microsatellite hitch-hiking selection in Atlantic cod (*Gadus morhua* L.): implications for inferring population structure in nonmodel organisms. *Molecular Ecology*, **15**, 3219–3229.
- Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D (2009a) Population genomics of marine fishes: identifying adaptive variation in space and time. *Molecular Ecology*, **18**, 3128–3150.
- Nielsen EE, Hemmer-Hansen J, Poulsen NA *et al.* (2009b) Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). *BMC Evolutionary Biology*, **9**, 276.
- Nielsen EE, MacKenzie BR, Magnussen E, Meldrup D (2007) Historical analysis of *Pan I* in Atlantic cod (*Gadus morhua*): temporal stability of allele frequencies in the southeastern part of the species distribution. *Canadian Journal of Fisheries and Aquatic Sciences*, **64**, 1448–1455.
- Nielsen R (2001) Statistical tests of selective neutrality in the age of genomics. *Heredity*, **86**, 641–647.
- Nielsen R (2005) Molecular signatures of natural selection. *Annual Review of Genetics*, **39**, 197–218.

- Nielsen R (2009) Adaptionism-30 years after Gould and Lewontin. *Evolution*, **63**, 2487–2490.
- O Leary DB, Coughlan J, Dillane E, McCarthy TV, Cross TF (2007) Microsatellite variation in cod *Gadus morhua* throughout its geographic range. *Journal of Fish Biology*, **70**, 310–335.
- Olsen EM, Moland E (2011) Fitness landscape of Atlantic cod shaped by harvest selection and natural selection. *Evolutionary Ecology*, **25**, 695–710.
- Olsen EM, Knutsen H, Gjøsæter J *et al.* (2008) Small-scale biocomplexity in coastal Atlantic cod supporting a Darwinian perspective on fisheries management. *Evolutionary Applications*, **1**, 524–533.
- Orsini L, Spanier KI, De Meester L (2012) Genomic signature of natural and anthropogenic stress in wild populations of the waterflea *Daphnia magna*: validation in space, time and experimental evolution. *Molecular Ecology*, **21**, 2160–2175.
- Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology*, **13**, 55–65.
- Palstra FP, Ruzzante DE (2008) Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Molecular Ecology*, **17**, 3428–3447.
- Palumbi SR (2001) Humans as the world's greatest evolutionary force. *Science*, **293**, 1786–1790.
- Pampoulie C, Ruzzante DE, Chosson V *et al.* (2006) The genetic structure of Atlantic cod (*Gadus morhua*) around Iceland: insight from microsatellites, the *Pan I* locus, and tagging experiments. *Canadian Journal of Fisheries and Aquatic Sciences*, **63**, 2660–2674.
- Pampoulie C, Stefansson MO, Jorundsdottir TD, Danilowicz BS, Danielsdottir AK (2008) Recolonization history and large-scale dispersal in the open sea: the case study of the North Atlantic cod, *Gadus morhua* L. *Biological Journal of the Linnean Society*, **94**, 315–329.
- Pardoe H, Vainikka A, Thordarson G, Marteinsdottir G, Heino M (2009) Temporal trends in probabilistic maturation reaction norms and growth of Atlantic cod (*Gadus morhua*) on the Icelandic shelf. *Canadian Journal of Fisheries and Aquatic Sciences*, **66**, 1719–1733.
- Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 637–669.
- Pauly D, Christensen V, Guenette S *et al.* (2002) Towards sustainability in world fisheries. *Nature*, **418**, 689–695.
- Pespeni MH, Garfield DA, Manier MK, Palumbi SR (2012) Genome-wide polymorphisms show unexpected targets of natural selection. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **279**, 1412–1420.
- Poulsen NA, Nielsen EE, Schierup MH, Loeschcke V, Grønkjær P (2006) Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). *Molecular Ecology*, **15**, 321–331.
- Poulsen N, Hemmer-Hansen J, Loeschcke V (2011) Microgeographical population structure and adaptation in Atlantic cod *Gadus morhua*. *Marine Ecology Progress Series*, **436**, 231–243.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Rasmussen M, Li Y, Lindgreen S *et al.* (2010) Ancient human genome sequence of an extinct Palaeo-Eskimo. *Nature*, **463**, 757–762.
- Rätz H, Lloret J (2005) Long-term variability of growth and recruitment of cod (*Gadus morhua*) off Greenland. *ICES Journal of Marine Science*, **62**, 1310–1321.
- Reed DH, Frankham R (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution*, **55**, 1095–1103.
- Reynolds JD, Dulvy NK, Goodwin NB, Hutchings JA (2005) Biology of extinction risk in marine fishes. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **272**, 2337–2344.
- Reznick DN, Ghalambor CK (2005) Can commercial fishing cause evolution? Answers from guppies (*Poecilia reticulata*). *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 791–801.
- Riccioni G, Landi M, Ferrara G *et al.* (2010) Spatio-temporal population structuring and genetic diversity retention in depleted Atlantic bluefin tuna of the Mediterranean Sea. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 2102–2107.
- Robichaud D, Rose GA (2004) Migratory behaviour and range in Atlantic cod: inference from a century of tagging. *Fish and Fisheries*, **5**, 185–214.
- Root TL, Price JT, Hall KR *et al.* (2003) Fingerprints of global warming on wild animals and plants. *Nature*, **421**, 57–60.
- Rosenblum EB, Novembre J (2007) Ascertainment bias in spatially structured populations: a case study in the eastern fence lizard. *The Journal of heredity*, **98**, 331–336.
- Ruzzante DE, Taggart CT, Doyle RW, Cook D (2001) Stability in the historical pattern of genetic structure of Newfoundland cod (*Gadus morhua*) despite the catastrophic decline in population size from 1964 to 1994. *Conservation Genetics*, **2**, 257–269.
- Ryman N, Utter F, Laikre L (1995) Protection of intraspecific biodiversity of exploited fishes. *Reviews in Fish Biology and Fisheries*, **5**, 417–446.
- Salinas S, Perez KO, Duffy TA *et al.* in press. The response of correlated traits following cessation of fishery-induced selection. *Evolutionary Applications*, **Early View: Article first published online: 15 feb 2012**.
- Sambatti JBM, Rice KJ (2006) Local adaptation, patterns of selection, and gene flow in the Californian serpentine sunflower (*Helianthus exilis*). *Evolution*, **60**, 696–710.
- Schindler DE, Hilborn R, Chasco B *et al.* (2010) Population diversity and the portfolio effect in an exploited species. *Nature*, **465**, 609–612.

- Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution*, **22**, 25–33.
- Siepielski AM, DiBattista JD, Evans JA, Carlson SM (2011) Differences in the temporal dynamics of phenotypic selection among fitness components in the wild. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **278**, 1572–1580.
- Slate J, Gratten J, Beraldi D *et al.* (2008) Gene mapping in the wild with SNPs: guidelines and future directions. *Genetica*, **136**, 97–107.
- Smith TB, Bernatchez L (2008) Evolutionary change in human-altered environments. *Molecular Ecology*, **17**, 1–8.
- Star B, Nederbragt AJ, Jentoft S *et al.* (2011) The genome sequence of Atlantic cod reveals a unique immune system. *Nature*, **477**, 207–210.
- Stinchcombe JR, Hoekstra HE (2007) Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. *Heredity*, **100**, 158–170.
- Stockwell CA, Hendry AP, Kinnison MT (2003) Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution*, **18**, 94–101.
- Stokes TK, Law R (2000) Fishing as an evolutionary force. *Marine Ecology Progress Series*, **208**, 307–309.
- Storz JF (2005) Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology*, **14**, 671–688.
- Struebig MJ, Kingston T, Petit EJ *et al.* (2011) Parallel declines in species and genetic diversity in tropical forest fragments. *Ecology Letters*, **14**, 582–590.
- Svedäng H, Righton D (2007) Migratory behaviour of Atlantic cod *Gadus morhua*: natal homing is the prime stock-separating mechanism. *Marine Ecology Progress Series*, **345**, 1–12.
- Svensson EM, Anderung C, Baubliene J *et al.* (2007) Tracing genetic change over time using nuclear SNPs in ancient and modern cattle. *Animal Genetics*, **38**, 378–383.
- Swain DP, Sinclair AF, Mark Hanson J (2007) Evolutionary response to size-selective mortality in an exploited fish population. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **274**, 1015–1022.
- Szulkin M, Bierne N, David P (2010) Heterozygosity-fitness correlations: a time for reappraisal. *Evolution*, **64**, 1202–1217.
- Tallmon DA, Luikart G, Beaumont MA (2004) Comparative evaluation of a new effective population size estimator based on approximate bayesian computation. *Genetics*, **167**, 977–988.
- Therkildsen NO, Nielsen EE, Swain DP, Pedersen JS (2010) Large effective population size and temporal genetic stability in Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 1585–1595.
- Thornton KR, Jensen JD (2007) Controlling the false-positive rate in multilocus genome scans for selection. *Genetics*, **175**, 737–750.
- Umina PA, Weeks AR, Kearney MR, McKechnie SW, Hoffmann AA (2005) A rapid shift in a classic clinal pattern in *Drosophila* reflecting climate change. *Science*, **308**, 691–693.
- Wandeler P, Hoeck PEA, Keller LF (2007) Back to the future: museum specimens in population genetics. *Trends in Ecology & Evolution*, **22**, 634–642.
- Wang J (2005) Estimation of effective population sizes from data on genetic markers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **360**, 1395–1409.
- Wang JL (2001) A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genetical Research*, **78**, 243–257.
- Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics*, **10**, 57–63.
- Waples RS (1989) A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics*, **121**, 379–391.
- Waples RS (1998) Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, **89**, 438–450.
- Waples RS (2002) Evaluating the effect of stage-specific survivorship on the N_e/N ratio. *Molecular Ecology*, **11**, 1029–1037.
- Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Notes*, **8**, 753–756.
- Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, **15**, 1419–1439.
- Watson CL, Lockwood DNJ (2009) Single nucleotide polymorphism analysis of European archaeological *M. leprae* DNA. *PLoS ONE*, **4**, e7547.
- Yamamichi M, Innan H (2012) Estimating the migration rate from genetic variation data. *Heredity*, **108**, 362–363.

CHAPTER 2

**Large effective population size and temporal genetic stability
in Atlantic cod (*Gadus morhua*) in the southern Gulf of St.
Lawrence**

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Large effective population size and temporal genetic stability in Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence

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Abstract: Worldwide, many commercial fish stocks have experienced dramatic declines due to overfishing. Such fisheries-induced population reductions could potentially erode the genetic diversity of marine fish populations. Based on analyses of DNA extracted from archived and contemporary samples, this paper compares the genetic variability at nine microsatellite loci in a Canadian population of Atlantic cod (*Gadus morhua*) over 80 years, spanning from before the fishery intensified to now when the population is at historically low abundance. Extensively validated genetic data from the temporally spaced samples were used to estimate the effective population size. Over the period, we observed no loss of either heterozygosity or allelic diversity. Several of the estimation methods applied could not distinguish the effective population size from infinity, and the lower 95% confidence limit on estimates was generally >500, suggesting that the effective population size is probably considerably larger than this. Hence, it appears that the southern Gulf of St. Lawrence cod stock has maintained genetic variability to sustain future evolution despite a dramatic population decline.

Introduction

In recent years many commercial fish stocks have declined dramatically as a result of overfishing (Hutchings and Reynolds 2004; Worm et al. 2006). A growing body of literature has addressed the possible genetic consequences of such extensive fishing pressure in relation to selection on ecologically and demographically important traits such as growth and age and size at maturity (e.g., Stokes and Law 2000; Conover and Munch 2002; Law 2007). Meanwhile, there has generally been less focus on whether severe

fisheries-induced population reductions have eroded the genetic diversity of exploited stocks.

The general lack of concern for this issue probably relates to the very large sizes of most exploited fish populations. Even after commercial collapse, these populations number millions of individuals. This is well above the population sizes typically considered at risk of losing genetic diversity (Frankham et al. 2002 and references therein). However, the rate of loss of genetic diversity is determined by the effective population size (N_e) rather than the census size (N), and several studies have suggested that the ratio between these

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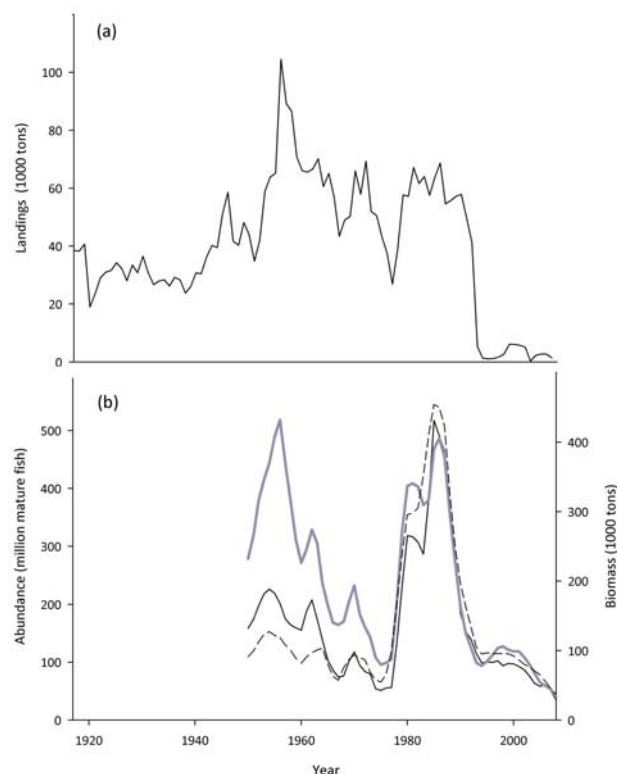
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two parameters (the N_e/N ratio) could be extremely low in marine fish compared with natural populations of most other organisms (see review by Hauser and Carvalho 2008). Surprisingly small effective population sizes of hundreds to a few thousand individuals have been reported for marine fish populations whose census sizes are up to five orders of magnitude larger (see e.g., Hauser et al. 2002; Hutchinson et al. 2003; Hoarau et al. 2005).

Although the degree to which such extreme estimates of N_e/N ratios reflect the true dynamics of biological systems has been questioned (Flowers et al. 2002; Poulsen et al. 2006), these findings caution that marine fish could be at greater risk of losing genetic diversity because of fisheries-induced population reductions than previously believed. Apparent loss of genetic diversity following intensive exploitation has already been reported for several marine fish species (Smith et al. 1991; Hauser et al. 2002; Hutchinson et al. 2003), but other studies have found temporal stability in genetic diversity despite heavy fishing pressure (Ruzzante et al. 2001; Jakobsdóttir et al. 2006; Poulsen et al. 2006). It is therefore important that we gain a better understanding of how vulnerable fish stocks are to erosion of genetic variation.

To this end, we studied temporal genetic variation in Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence, Canada. This population has been exploited commercially for centuries, but fishing intensified in the 1940s, with landings peaking at >100 000 t in 1956 (Fisheries and Oceans Canada (DFO) 2008; Fig. 1a). Biomass and abundance of commercial-sized cod declined sharply in the 1960s and early 1970s, but then quickly increased again (because of a period of unusually strong recruitment) (Fig. 1b). However, like many of the other Canadian cod stocks, the southern Gulf of St. Lawrence stock suffered a second dramatic decline in the early 1990s, and at that time a moratorium on fishing was imposed because of the low abundance. While the collapse in this area was not at first as dramatic as in some of the nearby stocks (e.g., the northern cod off Newfoundland; Hutchings and Reynolds 2004), the population has not yet recovered despite more than 15 years of severely reduced fishing pressure. During the early population reduction in the 1960s, abundance declined less sharply for mature individuals than for the total or exploitable population (Fig. 1b). This is because the age at maturity declined markedly during this period (Swain 2010), so that increased spawning at young ages partly compensated for the loss of older spawners and the mean age of spawners substantially declined (Supplemental Fig. S1³). Thus, in addition to census size fluctuations, the population has undergone dramatic demographic changes, which could also affect the N_e , especially if reproductive success is correlated with body size. The estimated abundance of mature individuals is currently at the lowest level ever recorded. In 2008 the spawning stock was estimated to consist of about 44 million fish, about 8% of the recorded maximum and about one-third of the harmonic mean spawning stock size over the period 1950–2008 (118 million fish), and spawner abundance is expected to continue to decline (DFO 2008; Swain and Chouinard 2008).

Fig. 1. Historical landings (a) and estimated biomass and abundance (b) for the southern Gulf of St. Lawrence cod stock. Panel b shows mature abundance (dashed line) as well as biomass (heavy grey line) and abundance (solid black line) of cod aged 5 years and older (the main ages contributing to the fishery). Landings and estimates of abundance and biomass at age are from Chouinard et al. (2008); mature abundance is computed based on revised maturity ogives estimated by D.P. Swain (unpublished data).



Based on DNA extracted from archived and contemporary samples, we compared the genetic composition of this population over a time span of 80 years from before the fishery intensified to the present. We conducted an extensive quality control of the historical data because the degraded DNA from these samples makes genotyping particularly error-prone. The results were used to investigate whether this population has suffered a loss of genetic diversity following intensive exploitation. To gain a better understanding of the observed pattern, we also applied several methods to estimate the N_e . With an estimated mean census size of 118 million individuals, we expect that the N_e of this population could still be large, and it is notoriously difficult to distinguish a large N_e from a very large one based on genetic data (Nei and Tajima 1981; Waples 1989; Palstra and Ruzzante 2008). For this reason — and because it arguably is the most relevant indicator for precautionary management — we focused particularly on the lower limits of the confidence intervals of N_e estimates to assess whether the N_e of this population could be of conservation concern.

³ Supplementary data for this article are available on the journal Web site (<http://cjfas.nrc.ca>).

Material and methods

Samples

The source of historical DNA from the southern Gulf of St. Lawrence cod stock was a set of archived otoliths collected in 1928 near Paspébiac in the western end of the southern Gulf (Fig. 2). These otoliths ($n = 57$ individuals) were obtained from the National Institute of Aquatic Resources in Denmark and had been stored individually in paper envelopes at room temperature. Contemporary samples ($n = 60$ individuals) were obtained from gill tissue collected within <100 km of the historical sampling location from mature individuals at spawning time (May–June) in 2008.

Laboratory procedures

DNA was extracted from both historical and contemporary samples with an Omega EZNA Tissue DNA kit (Omega Bio-Tek, USA). This method was used because it has been demonstrated to perform well with otolith DNA samples and does not damage otoliths during extraction (Therkildsen et al. 2010). The gill tissue was processed according to the manufacturer's instructions. Otoliths were left in the digestion solution for 3 h and removed before DNA was purified from the extract following the same procedure as used for gill tissue.

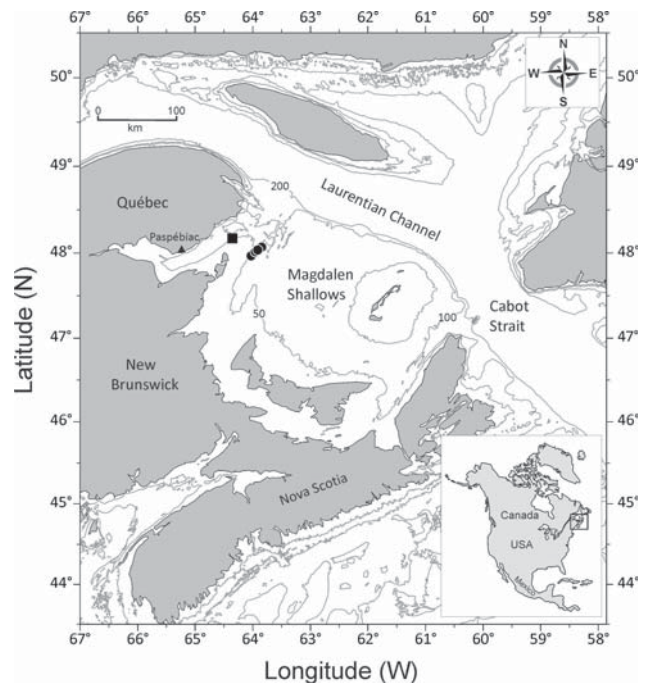
The samples were genotyped at nine di-, tri-, and tetra-nucleotide microsatellite loci: *Gmo 2* (di-) and *Gmo 132* (di-) (Brooker et al. 1994); *Gmo 8* (tetra-), *Gmo 19* (tetra-), *Gmo 34* (tetra-), and *Gmo 35* (tri-) (Miller et al. 2000); *Tch 11* (tetra-) and *Tch 14* (tetra-) (O'Reilly et al. 2000); and *Gadm 1* (di-) (Hutchinson et al. 2001) (see Table 1 for allele size ranges). For the contemporary samples, polymerase chain reaction (PCR) amplification was conducted using 30 PCR cycles, while historical samples were amplified with 39 cycles. The PCR amplification products were analyzed on a Basestation51 automated sequencer (MJ Research/Bio-Rad), and allele sizes were scored using the CARTOGRAPHER Sequencing and Genotyping Analysis Software (MJ Research). Individuals with known genotypes were included in all gel runs to ensure correct fragment size scoring.

Quality control of the genotyping

Because DNA in historical samples is degraded and only available in small quantities, it is more prone to generating genotyping errors than DNA from contemporary samples (Taberlet et al. 1996; Pompanon et al. 2005). Therefore, several precautions were taken to minimize this risk. Both DNA extraction and PCR preparation with the historical samples were conducted in an isolated laboratory located in a separate building where no fish samples had previously been analyzed. Negative controls were used in all runs (both extraction and PCR). Furthermore, all historical samples were genotyped following a multiple tubes approach (Navidi et al. 1992; Taberlet et al. 1996) based on generating several independent replicates of each genotype, because consensus genotypes (consistent results from multiple reactions) should be more reliable than results from a single reaction.

Two amplifications were initially carried out for each individual at each locus. If the same genotype was obtained in both, this consensus genotype was recorded. If different genotypes were observed in the replicates, additional PCRs were conducted until each allele had been observed twice or a

Fig. 2. The southern Gulf of St. Lawrence, showing sampling locations for the 1928 sample (square) and the 2008 sample (circles). The 50, 100, and 200 m isobaths are indicated by the grey lines.



consensus homozygote genotype could be assigned. In cases where three or more replicate genotypes were identical but a single replicate was different from the others, we followed the methodology in Miller and Waits (2003) and devised the consensus genotype based on the principle of parsimony. Initial trials had shown that the two types of genotyping error — allelic dropout (non-amplification of one allele) and mistaken alleles (mismatch between assigned alleles and consensus genotype due to, e.g., PCR artifacts, mis-scoring, other human errors, or sporadic contamination) — were almost equally common. Hence, in an example of four replicates yielding genotypes AA, AA, AA, and AB, the most parsimonious consensus genotype would be a homozygote with one mistaken allele rather than a heterozygote where the same allele had dropped out three times. In cases where a consensus genotype could not be assigned after seven independent PCRs or where more than four amplification attempts had failed, the genotype was recorded as missing. Only consensus genotypes were used for further analyses. To assess the repeatability of results, >35% of genotypes (including all homozygotes at most loci) from the contemporary samples were also replicated in independent PCRs.

Genotyping error rates were assessed by recording mismatches between replicates and corresponding consensus genotypes (assuming the latter represented “true” genotypes). Allelic dropouts and mistaken alleles were recorded independently. We quantified errors both per amplified allele (as defined by Broquet and Petit (2004)) and the proportion of positive PCRs that contained at least one of the two error types (as suggested by Hoffman and Amos 2005; Pompanon et al. 2005). Finally, to identify genotyping errors not

Table 1. Sample size (n), allelic richness (adjusted to 39 individuals), observed (H_o) and expected (H_e) heterozygosity, p value for the tests of deviation from Hardy–Weinberg Equilibrium (HWE), and locus-specific F_{ST} for the two temporally spaced samples from southern Gulf of St. Lawrence cod.

Locus	Size range (bp)	n		Allelic richness		H_o		H_e		p (HWE)		F_{ST}
		1928	2008	1928	2008	1928	2008	1928	2008	1928	2008	
<i>Gmo 2</i>	107–153	46	60	12.5	12.3	0.87	0.77	0.83	0.81	0.44	0.26	0.005
<i>Gmo 8</i>	121–289	46	60	13.7	16.6	0.89	0.88	0.92	0.92	0.13	0.84	0.001
<i>Gmo 19</i>	120–224	40	60	20.9	19.0	0.98	0.95	0.94	0.94	0.86	0.19	0.000
<i>Gmo 34</i>	095–111	49	60	4.8	4.6	0.65	0.43	0.57	0.43	0.93	0.16	0.016
<i>Gmo 35</i>	121–148	47	60	7.6	7.6	0.79	0.73	0.77	0.78	0.35	0.44	0.005
<i>Gmo 132</i>	111–125	47	60	6.0	6.5	0.79	0.58	0.70	0.63	0.67	0.17	0.009
<i>Tch 11</i>	115–215	39	60	18.0	20.0	0.92	0.92	0.93	0.94	0.32	0.54	0.001
<i>Tch 14</i>	103–231	45	60	19.3	19.3	0.96	0.87	0.94	0.93	0.14	0.42	–0.008
<i>Gadm 1</i>	168–180	45	60	5.9	5.6	0.62	0.55	0.62	0.59	0.29	0.21	–0.008
Average across loci		45	60	12.1	12.4	0.83	0.74	0.80	0.77	0.27*	0.25*	0.002*

*Results from multi-locus test or estimation.

detectable through replication (see DeWoody et al. 2006), the program MICRO-CHECKER (Van Oosterhout et al. 2004) was used to test for evidence of stutter, null alleles, and large allele dropout in the consensus data sets for both 1928 and 2008.

Testing assumptions for the statistical analysis

Deviations from Hardy–Weinberg proportions for each locus and sample as well as genotypic disequilibrium between all pairs of loci in each sample were tested using the program FSTAT version 2.9.3.2 (Goudet 1995, 2001). A sequential Bonferroni correction was applied to correct for multiple tests (Rice 1989). In addition, to test for evidence of selection effects on any locus, a neutrality test was applied using the Bayesian method implemented in the software BAYESCAN (Foll and Gaggiotti 2008).

Comparison of genetic diversity

Using FSTAT version 2.9.3.2 (Goudet 1995, 2001), the genetic diversity of each sample was quantified by Nei's (1987) unbiased heterozygosity and by allelic richness (adjusted to 39 individuals, which was the smallest number of individuals genotyped for any locus; El Mousadik and Petit 1996). To test for differences in diversity between samples, these statistics were computed for each locus in each sample and were compared by a Wilcoxon's signed rank test. We used the χ^2 test implemented in the program CHIFISH (Ryman 2006) to test for differences in allele frequency distributions at any locus between the samples. The genetic differentiation between samples was also estimated by single- and multi-locus F_{ST} values following Weir and Cockerham (1984). The 95% confidence interval on the multilocus estimate was determined by bootstrapping over loci, and the significance was tested by permuting genotypes between samples using the test procedure by Goudet et al. (1996) and 1000 permutations as implemented in FSTAT version 2.9.3.2 (Goudet 1995, 2001).

Estimation of the effective population size: single sample estimator

We applied the linkage disequilibrium (LD) method to obtain separate estimates of N_e in 1928 and in 2008. This method is based on the notion that LD at neutral, unlinked

loci in isolated randomly mating populations should arise only as a result of drift, and hence the level of nonrandom association of alleles at different loci should reflect the N_e of the population (Waples 2006; Waples and Do 2009). We estimated N_e with each sample using the program LDNE (Waples and Do 2008). Since allele frequencies close to 0 or 1 can affect this method in ways that are not completely understood (Waples 2006), we used a cutoff point at frequencies >0.02 (excluding single copy alleles), which has been shown to minimize bias for simulated data sets similar to ours (Waples and Do 2009). Estimates of N_e were computed based on the entire data set, but to assess sample size effects when comparing the 2008 results with the 1928 sample, the analysis was repeated on a subset of 40 randomly selected individuals from 2008.

Estimation of the effective population size: the temporal method

The temporal method for estimating N_e is based on quantifying the amount of drift or the rate of coalescence that has occurred between two or more samples taken at different times from the same population (Leberg 2005; Wang 2005). No universally superior estimator has been identified (Tallmon et al. 2004; Jorde and Ryman 2007), so we compared several different applications that are based on different mathematical properties. Common to these approaches is the assumption that mutation, migration, and selection have not significantly influenced the variance in allele frequencies between samples.

Estimating the generation length

Application of the temporal method to estimate N_e requires information about the number of generations between samples. The generation length of populations with overlapping generations is equal to the mean age of parents (Hill 1979). Following Miller and Kapuscinski (1997), this was approximated as the mean age of spawners weighted by age-specific fecundity. The relative fecundity of each female age class was calculated based on the stock-specific relationship between age and fecundity (McIntyre and Hutchings 2003). Abundance and maturity data on each age class (D.P. Swain, unpublished data) were then weighted by these relative fecundities. Although differences in reproductive

success among males have been demonstrated in captivity (Bekkevold et al. 2002), there are no data to quantify this effect in the wild. Therefore male reproductive contribution was assumed to be equal across age classes.

An average of the weighted female and unweighted male age distributions of spawners was computed for each year in the period 1950–2008 (Supplemental Fig. S1²). The overall average generation time in the period was 6.35 years, corresponding to 12.6 generations between the samples. This is probably a slight overestimate because the average age of spawners has declined during the period (Swain 2010; Supplemental Fig. S1²), and no data were available for 1928–1950 when it was likely to have been higher. Hence, for the analyses with the temporal method, we applied an estimated span of 12 generations. To assess the sensitivity of our N_e estimates to uncertainty in generation time, all analyses were also conducted assuming spans of 9 and 15 generations.

Moment estimator

The classical estimator of Waples (1989) is widely applied for estimating temporal N_e . However, Jorde and Ryman (2007) developed a modified, less biased moment estimator for small sample sizes and skewed allele frequencies (many rare alleles), as characterized in this data set. We used the program TEMPOFS (Jorde and Ryman 2007) to estimate N_e with this modified estimator.

Pseudo-likelihood estimator

In addition to the computationally simple moment estimator, we also used the pseudo-likelihood method of Wang (2001) as implemented in the program MNE 2 (Wang and Whitlock 2003). This method finds the value of N_e that maximizes the probability of the data based on a Wright–Fisher model of genetic drift and uses more information about allele frequencies in the samples than do moment-based estimators. The program requires an input for the maximum value that N_e may assume (N_{eMAX}), and we conducted separate analyses with an N_{eMAX} of 10^3 , 10^4 , and 3×10^4 (the largest value the program could handle on a standard computer).

ABC estimator

As a final application of the temporal method, N_e was estimated with approximate Bayesian computation (ABC) implemented in the program DIY ABC (Cornuet et al. 2008). Based on summary statistics known to have a relationship with N_e , this method compares the observed data set sampled from the studied population with an unknown N_e against 500 000 data sets sampled temporally from simulated populations, each with a known N_e . We used different sets of broad uniform priors on N_e ranging from 1 to 10^6 and a generalized stepwise mutation model with the prior parameters used in Cornuet et al. (2008). The posterior distribution for N_e was generated with a local logistic regression based on logit-transformed estimates of N_e from 5000 simulated data sets (the 1% most similar to the observed data). Three independent runs were conducted for each set of priors for N_e to check the consistency of results, and the presented results are averages of the mode and the 0.025 and 0.975 quartiles between these three runs.

Effect of genotyping error

To assess the potential effect of genotyping errors without quality control on our N_e estimates, all analyses were repeated with an uncorrected data set consisting of the first genotypes observed for each individual at each locus.

Results

Genotyping

Of the 57 historical individuals, eight were removed in a prescreening: three because they showed signs of multiple genotypes (pre-extraction sample contamination) and five because of poor amplification. For the remainder of the sample, on average 3.1 (range 2–7) positive PCRs were genotyped per locus per individual. The number of consensus genotypes obtained for the historical samples ranged from 39 to 49 per locus (Table 1). In single reactions, 7.9% of amplified alleles were classified as mistaken, and the average rate of allelic dropout was 8.9% (Supplemental Table S1²). The amplification success was higher for loci with shorter allele sizes (Supplemental Fig. S2²); thus, large allele dropout may have affected our historical data. Indeed, for six of the nine loci, the largest allele dropped out more often (data not shown). The effect of this on the consensus data appeared to be limited, however, as no significant differences in average allele size or allelic richness between the 1928 and the 2008 sample were detected (see below). Likewise, tests conducted in MICRO-CHECKER on the consensus data set did not detect evidence of large allele dropout, stutter, or null alleles.

The overall reliability of the consensus data can be coarsely approximated by calculating the probability of assigning a wrong allele to the consensus genotype after two independent reactions (i.e., the same error occurring in both replicates) as the squared probability of each type of error. Since average rates of mistaken alleles and allelic dropout were both around 8%–9%, this error is <0.8%. With additional replication (>2) for many genotypes, the actual error rate is likely even lower. Hence, despite the relatively high error rate for single amplifications, the overall reliability of the consensus data is likely above 99%.

All of the 60 contemporary samples were successfully genotyped at all loci. The replication of a subset of genotypes revealed an allelic dropout rate of 0.9% and 0.5% mistaken alleles, but these were corrected through replication.

Testing assumptions for the statistical analysis

The data showed no significant deviations from Hardy–Weinberg expectations for any locus in any sample (Table 1), and none of the pairwise comparisons in the linkage disequilibrium test were significant after Bonferroni correction. The neutrality test did not detect any outlier loci; the posterior probability of being affected by selection was £0.62 for all loci, and the results were consistent between independent runs (results not shown). Hence all loci were included in further analyses.

Comparison of genetic diversity

The average allelic richness was slightly higher in 2008 than in 1928, while the average expected heterozygosity was slightly higher in 1928 (Table 1). However, none of

these differences were significant on a locus-by-locus basis ($p > 0.17$ for both). There were also no significant differences in allele frequencies between the samples ($p = 0.51$), and the multilocus F_{ST} estimate was 0.002 (95% confidence interval: -0.002 to 0.006) and not significantly different from zero ($p = 0.48$).

Estimation of the effective population size

The results obtained with the different N_e estimators are summarized (Fig. 3). With the LD method, the point estimate for 1928 could not be distinguished from infinity. For 2008, the method yielded a finite estimate of 580 individuals (895 when the analysis was based on only 40 individuals), but the 95% confidence interval included infinity. While the point estimate for 2008 is smaller than that for 1928, the confidence intervals broadly overlap (even extending slightly lower in 2008 than in 1928; see Fig. 3), making the data inconclusive about possible changes in N_e over time. In both years, the lower bound of the confidence interval was around a few hundred.

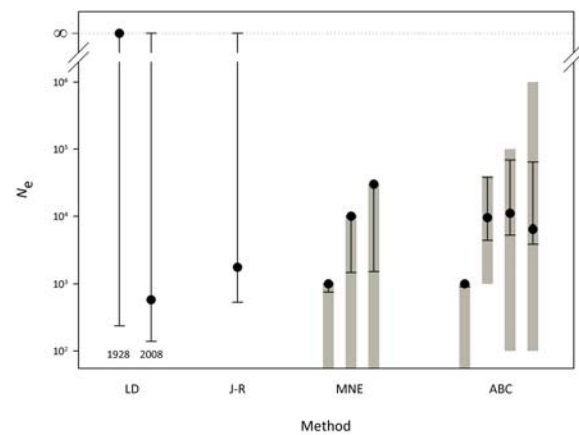
All three applications of the temporal method yielded somewhat larger point estimates. The Jorde–Ryman moment estimator provided a finite point estimate for N_e of 1768 with 95% confidence intervals spanning from just over 500 to infinity. The pseudo-likelihood-based estimator generated point estimates that were equal to or very close to the maximum allowed for all values of N_{eMAX} tested (Fig. 3), suggesting that N_e is $>3 \times 10^4$ (which was the largest size that could be tested here). The lower 95% confidence limit, however, was consistent between runs at around 10^3 regardless of the N_{eMAX} . The ABC method provided fairly consistent estimates of N_e for a broad range of prior values (Fig. 3), and it was the only method that yielded finite confidence intervals that were not constrained by the priors. When the maximum N_e allowed was $<10^4$ (the estimation process was limited to within the interval given in the priors), the estimate was exactly the maximum value, suggesting that N_e was larger than this. For all other prior sets tested, the estimate of N_e was around 10^4 , and lower and upper 95% confidence limits were around 5×10^3 to 6×10^4 , respectively. Results were similar for independent runs with the same priors.

Varying the assumed number of generations between samples affected the Jorde–Ryman and the MNE estimator in predictable ways. Reducing the span to nine generations caused a $\sim 25\%$ decrease in the lower confidence interval limit, while increasing it 15 generations caused a corresponding $\sim 25\%$ increase. The results obtained with the ABC method were more variable, but with lower confidence interval limits only changing by up to $\sim 10\%$. Based on available data for this population (Supplemental Fig. S1²), spans of 9 and 15 generations over the 80-year period appear to represent the extremes of plausible values. Thus these effects represent the maximum bias expected from uncertainty in estimating this parameter.

Effects of genotyping error

Analysis of the uncorrected data revealed large deviations from Hardy–Weinberg equilibrium (HWE) proportions at several loci in the 1928 sample and significant differences in allele frequencies between the two samples ($p = 0.03$). The average heterozygosity in both samples was identical to

Fig. 3. Point estimates (dots) and precision limits (error bars) of N_e obtained with different estimators. LD is the linkage disequilibrium method applied separately to the 1928 and the 2008 sample. J–R is the Jorde–Ryman temporal method, MNE is the pseudo-likelihood method, and ABC is the method based on approximate Bayesian computation. Error bars represent 95% confidence intervals (2.5% and 97.5% quartiles of the posterior distribution for the ABC). LD and J–R are moment-based and incorporate no prior. MNE uses a uniform prior from 0 to N_{eMAX} , and the ABC estimates are based on uniform priors from N_{eMIN} to N_{eMAX} . The priors used in different runs are illustrated by the shaded bars.



that computed for the consensus data set, while the average allelic richness was slightly lower both for the 1928 and the 2008 sample in the uncorrected data, though still not significantly different between samples ($p = 0.20$). The LD estimate based only on the 2008 sample was slightly higher with the uncorrected data, but all N_e estimates involving the historical sample (which was particularly error prone) were considerably lower with the uncorrected data (Supplemental Fig. S3²). Point estimates were at least 50% lower, and lower confidence interval limits were at least 35% lower than those obtained with the consensus data. The two methods for which point estimates were beyond the estimation capabilities with the consensus data (LD and MNE) also yielded finite estimates with uncorrected data (Supplemental Fig. S3²).

Discussion

Although a large reduction in census population size inevitably will result in the loss of many rare alleles at the genomic level (Ryman et al. 1995; Allendorf et al. 2008), our study suggests that the southern Gulf of St. Lawrence cod stock has not suffered any reduction in common genetic diversity indices at microsatellite loci despite severe population reductions due to intensive fishing over the past 80 years. The selection of methods applied to estimate the N_e varied in their point estimates and their precision, but generally had overlapping confidence intervals. The LD estimator suggested that N_e could be as low as a few hundred, but with a point estimate of infinity for the 1928 sample and exceedingly wide confidence intervals, this was also the most imprecise and uninformative estimator. All applications of the temporal method (based on more data using

both samples) suggested that N_e was larger than 500–5000 (lower 95% confidence interval limit). The consistent exclusion of very low values of N_e from the confidence intervals by these three methods that are based on different models with varying assumptions and different mathematical approaches provides compelling evidence that the N_e of this population is at the very least 500 and most likely considerably larger than that. This finding is consistent with the observation of temporal stability in genetic diversity because an N_e much above 500 exceeds the population sizes typically associated with immediate loss of genetic diversity due to drift (Frankham et al. 2002 and references therein).

Precision of estimates

The study remains inconclusive about the exact magnitude of N_e . The LD method was uninformative, with confidence intervals extending from a couple of hundred individuals to infinity. The point estimates from the temporal method indicated that it was either 2000 – 10 000 (Jorde–Ryman and ABC) or considerably greater (MNE). This uncertainty is not surprising given that in general, genetic N_e estimators do not perform well for large populations (Nei and Tajima 1981; Waples 1989) because when there is little drift, the signal-to-noise ratio becomes very small. This may compromise the accuracy of all the estimators, and it is not obvious which methods would perform best under such circumstances, as comparative simulation studies are typically only carried out for small to moderate N_e (see e.g., Tallmon et al. 2004; Jorde and Ryman 2007). In general, however, there are several ways that the precision of our estimates could have been improved: (i) increasing the number of individuals in each sample; (ii) increasing the number of generations between samples; (iii) integrating information from additional temporal samples; or (iv) increasing the number of loci or the variability of the loci used (Waples 1989; Wang 2001; Palstra and Ruzzante 2008).

Recent papers have emphasized that a very extensive sampling of individuals may be needed to achieve estimates with finite bounds when N_e is large (Ovenden et al. 2007; Palstra and Ruzzante 2008), but studies based on archived samples are limited by the number of historical individuals available and the extra time and resources required for their reliable genotyping. Although the sample size used in this study for this reason was modest, it is typical of studies using temporal sampling to estimate N_e (see Palstra and Ruzzante 2008). The finding of no genetic differentiation between temporal samples (with a narrow confidence interval of the F_{ST} estimate) also suggests that sampling effects were low. Reducing these sampling effects further (by increasing sample size) would thus likely have led to even smaller estimates of variance in allele frequencies, and hence larger sample sizes for this study could possibly have increased N_e estimates and narrowed confidence limits, but would unlikely have reduced the lower limit.

The relatively small sample size was also compensated for by using the oldest available samples providing the maximum number of generations between samples possible. With extensive resources for genotyping, multiple sampling times could have improved precision and accuracy and allowed inference about potential temporal changes in the N_e . However, if only a limited number of individuals can be

genotyped, information content is maximized by pursuing temporal extreme samples rather than spreading out the sampling within the time period studied.

While adding more loci may be the most straightforward way of increasing the power for N_e estimation, we expect that the number of loci analyzed would need to be considerably increased to achieve a markedly greater precision. Since we found no significant changes in allele frequencies at any of the loci studied, additional neutral loci are unlikely to show a significantly elevated drift signal. When this is the case, the estimators hardly have any temporal variance to work with — even with vast numbers of loci or samples. Accordingly, adding a few more loci is unlikely to add much power. A many-fold increase in the number of loci used may improve the statistical precision, but as pointed out by Palstra and Ruzzante (2008), the practical value of obtaining precise estimates of N_e when it is large may not always justify the extensive efforts required. While it may be of general interest to derive reliable inference about the N_e/N ratio in large populations, the main question of interest to management is whether N_e is small. It has been suggested that an N_e of up to 5000 may be required for maintaining evolutionary potential in the long term (e.g., Lande 1995). The ABC method indicated that N_e for the southern Gulf of St. Lawrence cod exceeded this value, but it was not excluded from the confidence intervals obtained with other methods. However, our estimates are in general sufficiently precise to demonstrate that the N_e of this population is unlikely to be below the more commonly considered critical size of 500 individuals (Frankham et al. 2002).

Comparison to N_e estimates in other studies

The N_e estimates presented here are similar in magnitude to those presented in several other studies on marine fish (e.g., Hoarau et al. 2005; Saillant and Gold 2006), but are much larger than the extremely small values of N_e of a few hundred that have been proposed for a North Sea cod population (Hutchinson et al. 2003) and for a New Zealand snapper stock (*Pagrus auratus*) (Hauser et al. 2002). One possible reason for the higher N_e estimates obtained in this study could simply be that the census size of the population is more than an order of magnitude larger than in the cited studies. If there were a fairly constant N_e/N ratio for marine fish populations, we would expect a proportionately larger effective size. However, the N_e/N ratio is likely to vary between populations depending on both environmental conditions and variation in life history parameters, so the discrepancies could reflect real population-specific differences. Nevertheless, it is also possible that the results presented here overestimate the true N_e or that results from other studies represent underestimates, as there are a range of factors that can affect N_e estimates, including violation of model assumptions and technical problems (e.g., through unidentified population structure, migration, genotyping errors, and inadequate sampling of individuals and loci).

Model assumptions

The temporal method is based on a model with discrete generations and assumes that mutation, selection, and migration are unimportant in changing population allelic frequencies relative to genetic drift. The assumption of discrete

generations is obviously violated because cod populations have overlapping generations. Using empirical data from the literature, Palstra and Ruzzante (2008) have confirmed that ignored age structure has probably introduced a strong bias into many empirical N_e estimates. However, simulations have shown that with about 12 generations between samples (as was the case here), the bias induced by this violation is negligible (Waples and Yokota 2007). The number of generations is probably also sufficiently low for effects of mutations to be safely ignored (Waples 1989; Beaumont 2003). However, the assumptions of no effects of selection on the markers used and of complete isolation of the studied population may be more problematic.

Microsatellites are generally expected to be neutral markers, and we did not detect evidence of temporal selection on any locus over the time span studied here. However, the power of this test was low because of the relatively small number of loci and samples (Guinand et al. 2004), and other studies have shown that two of the loci we employed (*Gmo 34* and *Gmo 132*) may be affected by selection (Nielsen et al. 2006; Westgaard and Fevolden 2007). In fact, these two loci showed the highest F_{ST} differentiation between samples, and although the changes in allele frequencies were not significant, the slightly larger differentiation could possibly indicate directional selection affecting these loci. This, in turn, generates larger variance in allele frequencies, resulting in smaller N_e estimates. Excluding *Gmo 34* and *Gmo 132* from analyses yielded point estimates and lower confidence interval limits 10%–65% higher than those based on all loci. Thus, including these two loci in our study could have biased the N_e estimates downwards, making our results conservative in terms of evaluating whether N_e could be small. Point estimates and lower confidence limits may for this reason be substantially larger than reported here.

In relation to migration, there are probably few completely isolated populations in nature, and this is especially true for marine fish that have long dispersal capabilities and live in an environment with few strong physical barriers to gene flow. Hence, the question is not whether the assumption of no gene flow is violated, but rather whether the effect is sufficiently small to safely be ignored. Migration can cause either an under- or an over-estimation of N_e depending on the degree of differentiation from source populations and the time scale involved (Wang and Whitlock 2003; Fraser et al. 2007). Migration from a dissimilar source can increase the change in allele frequencies between two temporally spaced samples, and if this is interpreted as a higher level of drift it will be translated to an underestimate of the N_e . Migration from a genetically similar source, on the other hand, may counteract the effects of drift by homogenizing gene frequencies between sampling times, and in such cases estimates of N_e could reflect the size of a larger metapopulation rather than the local population sampled (Wang and Whitlock 2003).

Given that this study detected virtually no signal of drift, only the latter scenario may be of concern. Based on the variation at six hypervariable microsatellites, Ruzzante et al. (2000) found that there was a significant genetic distance between cod samples within the Gulf of St. Lawrence and cod sampled from different nominal stocks in its approaches.

However, they did not find significant differentiation between the two nominal stocks within the Gulf, the southern and the northern stocks. Based on the available evidence, it cannot be excluded that gene flow from the northern Gulf has caused an overestimate of the effective size of the population residing in the southern Gulf.

However, both traditional tags and otolith elemental fingerprints as natural tags suggest a high degree of reproductive isolation between the cod in the northern and southern Gulf (Campana et al. 2000; Robichaud and Rose 2004). Further, there are significant differences in vertebral counts between the northern and southern populations (Swain et al. 2001). While this morphological trait is affected by environmental factors, it also has a genetic component and has been proposed to be fitness-related in other species (Billerbeck et al. 1997; Swain and Foote 1999). The difference may thus represent adaptive divergence between the populations. Such divergence was recently found between southern Gulf of St. Lawrence cod and other nearby populations, as there were genetically based differences in life history traits over scales with little differentiation at microsatellite loci (Hutchings et al. 2007). Therefore, the absence of differentiation at neutral markers between the southern and northern Gulf cod may reflect historical events and large effective population size rather than contemporary gene flow.

Undetected population substructure within the southern Gulf could also affect the spatial scale for which an N_e estimate applies. There is currently no genetic evidence available to suggest population structure within the southern Gulf itself, but should such structure exist, our N_e estimates would apply only to a portion of the nominal stock and hence underestimate the magnitude for the whole area.

Genotyping errors

In addition to violation of model assumptions, genotyping errors can also affect the N_e estimates. While probably few data sets on genetic markers are error free, there is a particular concern when working with historical material (Hoffman and Amos 2005; Pompanon et al. 2005). As demonstrated by our estimates based on uncorrected data, genotyping errors in the historical sample could lead to inflated measures of differentiation between temporal samples and hence marked underestimates of N_e . Despite our large efforts to minimize this effect, residual errors in our data set (arising from large allele dropout or other) may have biased our reported estimates downward. However, such errors are likely to cause departure from HWE, create signs of LD between loci, or cause divergence between samples, and none of these phenomena were observed. In combination with the failure of the software MICRO-CHECKER to detect deviations from expected frequency distributions of allele sizes and genotypes, this strongly suggests that genotyping error did not significantly influence our results.

Quantification of genotyping errors and incorporation of special precautions to mitigate their effect are standard procedures in most population genetic studies based on noninvasive samples (hair, feces, urine, etc.; Taberlet et al. 1999; Broquet and Petit 2004) and have been widely applied in studies using museum specimens as a source of DNA (e.g., Miller and Waits 2003; Sefc et al. 2003; Frantz et al. 2008). However, this issue is often not explicitly discussed in his-

torical genetic studies based on archived fish scales and otoliths. This is somewhat surprising given that this source of historical DNA may be available in smaller quantities and may be more susceptible to pre-extraction contamination than other sources of historical DNA, such as bone or teeth (because DNA is recovered from the surface of otoliths and hence samples cannot be decontaminated prior to extraction). The present study clearly demonstrates that genotyping errors, if not accounted for, can pose considerable problems when working with these kinds of samples. We therefore highly encourage explicit quantification of genotyping error rates and careful quality control of data in future studies with historical otolith and scale samples.

Implications

Given that in the current study, the genetic data were extensively validated and the assumptions of the N_e estimation methods appear to be met, our findings clearly suggest that the cod population in the southern Gulf of St. Lawrence has a large effective population size and has not suffered any loss of heterozygosity and allelic richness at neutral markers over the past 80 years. This suggests that the extremely small estimates of N_e reported in other studies do not appear to be a universal phenomenon for marine fish populations.

The large N_e of the southern Gulf of St. Lawrence cod stock implies that future evolution in this population is unlikely to be constrained by reduced genetic variability. It also implies, however, that selection will be an effective force in driving this future evolution, as selection is counteracted by drift to a lesser extent in large populations than in small ones (Frankham et al. 2002 and references therein). Considering that the N_e estimates obtained here may be greater than estimates reported for many other populations of marine fish, southern Gulf of St. Lawrence cod may be particularly vulnerable to effects of fisheries-induced selection (as well as other contemporary selection pressures), which may reduce the capacity of the population to demographically deal with environmental perturbations. The data presented here on temporal stability at neutral markers will serve as an important baseline for future evaluations of adaptive evolution at the DNA level.

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References

Allendorf, F.W., England, P.R., Luikart, G., Ritchie, P.A., and Ryman, N. 2008. Genetic effects of harvest on wild animal po-

- pulations. *Trends Ecol. Evol.* **23**(6): 327–337. doi:10.1016/j.tree.2008.02.008. PMID:18439706.
- Beaumont, M.A. 2003. Estimation of population growth or decline in genetically monitored populations. *Genetics*, **164**(3): 1139–1160. PMID:12871921.
- Bekkevold, D., Hansen, M.M., and Loeschcke, V. 2002. Male reproductive competition in spawning aggregations of cod (*Gadus morhua*, L.). *Mol. Ecol.* **11**(1): 91–102. doi:10.1046/j.0962-1083.2001.01424.x. PMID:11903907.
- Billerbeck, J.N., Orti, G., and Conover, D.O. 1997. Latitudinal variation in vertebral number has a genetic basis in the Atlantic silverside, *Menidia menidia*. *Can. J. Fish. Aquat. Sci.* **54**(8): 1796–1801. doi:10.1139/cjfas-54-8-1796.
- Brooker, A.L., Cook, D., Bentzen, P., Wright, J.M., and Doyle, R.W. 1994. Organization of microsatellites differs between mammals and cold-water teleost fishes. *Can. J. Fish. Aquat. Sci.* **51**(9): 1959–1966. doi:10.1139/f94-198.
- Broquet, T., and Petit, E. 2004. Quantifying genotyping errors in noninvasive population genetics. *Mol. Ecol.* **13**(11): 3601–3608. doi:10.1111/j.1365-294X.2004.02352.x. PMID:15488016.
- Campana, S.E., Chouinard, G.A., Hanson, J.M., Frechet, A., and Brattey, J. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. *Fish. Res.* **46**(1–3): 343–357. doi:10.1016/S0165-7836(00)00158-2.
- Chouinard, G.A., Savoie, L., Swain, D.P., Hurlbut, T., and Daigle, D. 2008. Assessment of the southern Gulf of St. Lawrence cod stock, February 2008. DFO Can. Sci. Advis. Sec. Res. Doc. 2009/037.
- Conover, D.O., and Munch, S.B. 2002. Sustaining fisheries yields over evolutionary time scales. *Science (Washington, D.C.)*, **297**(5578): 94–96. doi:10.1126/science.1074085. PMID:12098697.
- Cornuet, J.M., Santos, F., Beaumont, M.A., Robert, C.P., Marin, J.M., Balding, D.J., Guillemaud, T., and Estoup, A. 2008. Inferring population history with DIYABC: a user-friendly approach to approximate Bayesian computation. *Bioinformatics*, **24**(23): 2713–2719. doi:10.1093/bioinformatics/btn514. PMID:18842597.
- Department of Fisheries and Oceans. 2008. Assessment of cod in the southern Gulf of St. Lawrence. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2008/004 (revised).
- DeWoody, J., Nason, J.D., and Hipkins, V.D. 2006. Mitigating scoring errors in microsatellite data from wild populations. *Mol. Ecol. Notes*, **6**(4): 951–957. doi:10.1111/j.1471-8286.2006.01449.x.
- El Mousadik, A., and Petit, R.J. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theor. Appl. Genet.* **92**(7): 832–839. doi:10.1007/BF00221895.
- Flowers, J.M., Schroeter, S.C., and Burton, R.S. 2002. The recruitment sweepstakes has many winners: genetic evidence from the sea urchin *Strongylocentrotus purpuratus*. *Evolution*, **56**(7): 1445–1453. PMID:12206244.
- Foll, M., and Gaggiotti, O. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics*, **180**(2): 977–993. doi:10.1534/genetics.108.092221. PMID:18780740.
- Frankham, R., Ballou, J.D., and Briscoe, D.A. 2002. Introduction to conservation genetics. Cambridge University Press, Cambridge, UK.
- Frantz, A.C., Hamann, J.L., and Klein, F. 2008. Fine-scale genetic structure of red deer (*Cervus elaphus*) in a French temperate forest. *Eur. J. Wildl. Res.* **54**(1): 44–52. doi:10.1007/s10344-007-0107-1.
- Fraser, D.J., Hansen, M.M., Østergaard, S., Tessier, N., Legault, M., and Bernatchez, L. 2007. Comparative estimation

- of effective population sizes and temporal gene flow in two contrasting population systems. *Mol. Ecol.* **16**(18): 3866–3889. doi:10.1111/j.1365-294X.2007.03453.x. PMID:17850551.
- Goudet, J. 1995. FSTAT (Version 1.2): a computer program to calculate F-statistics. *J. Hered.* **86**(6): 485–486.
- Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (Version 2.9.3). Available from <http://www2.unil.ch/popgen/softwares/fstat.htm> [accessed 1 October 2008; updated 23 August 2005].
- Goudet, J., Raymond, M., de Meeüs, T., and Rousset, F. 1996. Testing differentiation in diploid populations. *Genetics*, **144**(4): 1933–1940. PMID:8978076.
- Guinand, B., Lemaire, C., and Bonhomme, F. 2004. How to detect polymorphisms undergoing selection in marine fishes? A review of methods and case studies, including flatfishes. *J. Sea Res.* **51**(3–4): 167–182. doi:10.1016/j.seares.2003.10.002.
- Hauser, L., and Carvalho, G.R. 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. *Fish Fish.* **9**(4): 333–362.
- Hauser, L., Adcock, G.J., Smith, P.J., Ramiréz, J.H.B., and Carvalho, G.R. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proc. Natl. Acad. Sci. U.S.A.* **99**(18): 11742–11747. doi:10.1073/pnas.172242899. PMID:12185245.
- Hill, W.G. 1979. A note on effective population size with overlapping generations. *Genetics*, **92**(1): 317–322. PMID:17248921.
- Hoarau, G., Boon, E., Jongma, D.N., Ferber, S., Pálsson, J., Van der Veer, H.W., Rijnsdorp, A.D., Stam, W.T., and Olsen, J.L. 2005. Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **272**(1562): 497–503. doi:10.1098/rspb.2004.2963.
- Hoffman, J.I., and Amos, W. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Mol. Ecol.* **14**(2): 599–612. doi:10.1111/j.1365-294X.2004.02419.x. PMID:15660949.
- Hutchings, J.A., and Reynolds, J.D. 2004. Marine fish population collapses: consequences for recovery and extinction risk. *Bioscience*, **54**(4): 297–309. doi:10.1641/0006-3568(2004)054[0297:MFPCCF]2.0.CO;2.
- Hutchings, J.A., Swain, D.P., Rowe, S., Eddington, J.D., Puvanendran, V., and Brown, J.A. 2007. Genetic variation in life-history reaction norms in a marine fish. *Proc. Biol. Sci.* **274**(1619): 1693–1699. doi:10.1098/rspb.2007.0263. PMID:17490948.
- Hutchinson, W.F., Carvalho, G.R., and Rogers, S.I. 2001. Marked genetic structuring in localised spawning populations of cod *Gadus morhua* in the North Sea and adjoining waters, as revealed by microsatellites. *Mar. Ecol. Prog. Ser.* **223**: 251–260. doi:10.3354/meps223251.
- Hutchinson, W.F., van Oosterhout, C., Rogers, S.I., and Carvalho, G.R. 2003. Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **270**(1529): 2125–2132. doi:10.1098/rspb.2003.2493.
- Jakobsdóttir, K.B., Danielsdóttir, A.K., Marteinsdóttir, G., and Pampoulié, C. 2006. Long-term temporal genetic analysis of spawning cod (*Gadus morhua*) in coastal waters SW of Iceland. *J. Fish Biol.* **69**(Suppl. C): 247–247.
- Jorde, P.E., and Ryman, N. 2007. Unbiased estimator for genetic drift and effective population size. *Genetics*, **177**(2): 927–935. doi:10.1534/genetics.107.075481. PMID:17720927.
- Lande, R. 1995. Mutation and conservation. *Conserv. Biol.* **9**(4): 782–791. doi:10.1046/j.1523-1739.1995.09040782.x.
- Law, R. 2007. Fisheries-induced evolution: present status and future directions. *Mar. Ecol. Prog. Ser.* **335**: 271–277. doi:10.3354/meps335271.
- Leberg, P. 2005. Genetic approaches for estimating the effective size of populations. *J. Wildl. Manag.* **69**(4): 1385–1399. doi:10.2193/0022-541X(2005)69[1385:GAFETE]2.0.CO;2.
- McIntyre, T.M., and Hutchings, J.A. 2003. Small-scale temporal and spatial variation in Atlantic cod (*Gadus morhua*) life history. *Can. J. Fish. Aquat. Sci.* **60**(9): 1111–1121. doi:10.1139/f03-090.
- Miller, L.M., and Kapuscinski, A.R. 1997. Historical analysis of genetic variation reveals low effective population size in a northern pike (*Esox lucius*) population. *Genetics*, **147**(3): 1249–1258. PMID:9383067.
- Miller, C.R., and Waits, L.P. 2003. The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implications for conservation. *Proc. Natl. Acad. Sci. U.S.A.* **100**(7): 4334–4339. doi:10.1073/pnas.0735531100. PMID:12655042.
- Miller, K.M., Le, K.D., and Beacham, T.D. 2000. Development of tri- and tetranucleotide repeat microsatellite loci in Atlantic cod (*Gadus morhua*). *Mol. Ecol.* **9**(2): 238–239. doi:10.1046/j.1365-294x.2000.00804-2.x. PMID:10672170.
- Navidi, W., Arnheim, N., and Waterman, M.S. 1992. A multiplex approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. *Am. J. Hum. Genet.* **50**(2): 347–359. PMID:1734715.
- Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.
- Nei, M., and Tajima, F. 1981. Genetic drift and estimation of effective population size. *Genetics*, **98**(3): 625–640. PMID:17249104.
- Nielsen, E.E., Hansen, M.M., and Meldrup, D. 2006. Evidence of microsatellite hitch-hiking selection in Atlantic cod (*Gadus morhua* L.): implications for inferring population structure in non-model organisms. *Mol. Ecol.* **15**(11): 3219–3229. doi:10.1111/j.1365-294X.2006.03025.x. PMID:16968266.
- O'Reilly, P.T., Canino, M.F., Bailey, K.M., and Bentzen, P. 2000. Isolation of twenty low stutter di- and tetranucleotide microsatellites for population analyses of walleye pollock and other gadoids. *J. Fish Biol.* **56**(5): 1074–1086. doi:10.1111/j.1095-8649.2000.tb02124.x.
- Ovenden, J.R., Peel, D., Street, R., Courtney, A.J., Hoyle, S.D., Peel, S.L., and Podlich, H. 2007. The genetic effective and adult census size of an Australian population of tiger prawns (*Penaeus esculentus*). *Mol. Ecol.* **16**(1): 127–138. doi:10.1111/j.1365-294X.2006.03132.x. PMID:17181726.
- Palstra, F.P., and Ruzzante, D.E. 2008. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Mol. Ecol.* **17**(15): 3428–3447. doi:10.1111/j.1365-294X.2008.03842.x. PMID:19160474.
- Pompanon, F., Bonin, A., Bellemain, E., and Taberlet, P. 2005. Genotyping errors: causes, consequences and solutions. *Nat. Rev. Genet.* **6**(11): 846–859. doi:10.1038/nrg1707. PMID:16304600.
- Poulsen, N.A., Nielsen, E.E., Schierup, M.H., Loeschcke, V., and Grønkrjaer, P. 2006. Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). *Mol. Ecol.* **15**(2): 321–331. doi:10.1111/j.1365-294X.2005.02777.x. PMID:16448403.
- Rice, W.R. 1989. Analysing tables of statistical tests. *Evolution*, **43**(1): 223–225. doi:10.2307/2409177.
- Robichaud, D., and Rose, G.A. 2004. Migratory behaviour and range in Atlantic cod: inference from a century of tagging. *Fish Fish.* **5**(3): 185–214.

- Ruzzante, D.E., Taggart, C.T., Lang, S., and Cook, D. 2000. Mixed-stock analysis of Atlantic cod near the Gulf of St. Lawrence based on microsatellite DNA. *Ecol. Appl.* **10**(4): 1090–1109. doi:10.1890/1051-0761(2000)010[1090:MSAOAC]2.0.CO;2.
- Ruzzante, D.E., Taggart, C.T., Doyle, R.W., and Cook, D. 2001. Stability in the historical pattern of genetic structure of Newfoundland cod (*Gadus morhua*) despite the catastrophic decline in population size from 1964 to 1994. *Conserv. Genet.* **2**(3): 257–269. doi:10.1023/A:1012247213644.
- Ryman, N. 2006. CHIFISH: a computer program testing for genetic heterogeneity at multiple loci using chi-square and Fisher's exact test. *Mol. Ecol. Notes*, **6**(1): 285–287. doi:10.1111/j.1471-8286.2005.01146.x.
- Ryman, N., Utter, F., and Laikre, L. 1995. Protection of intraspecific biodiversity of exploited fishes. *Rev. Fish Biol. Fish.* **5**(4): 417–446. doi:10.1007/BF01103814.
- Saillant, E., and Gold, J.R. 2006. Population structure and variance effective size of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. *Fish. Bull. (Washington, D.C.)*, **104**(1): 136–148.
- Sefc, K.M., Payne, R.B., and Sorenson, M.D. 2003. Microsatellite amplification from museum feather samples: effects of fragment size and template concentration on genotyping errors. *Auk*, **120**(4): 982–989. doi:10.1642/0004-8038(2003)120[0982:MAFMFS]2.0.CO;2.
- Smith, P.J., Francis, R., and McVeagh, M. 1991. Loss of genetic diversity due to fishing pressure. *Fish. Res.* **10**(3–4): 309–316. doi:10.1016/0165-7836(91)90082-Q.
- Stokes, T.K., and Law, R. 2000. Fishing as an evolutionary force. *Mar. Ecol. Prog. Ser.* **208**: 307–309.
- Swain, D.P. 2010. Life-history evolution and elevated natural mortality in a population of Atlantic cod (*Gadus morhua*). *Evol. Appl.* doi:10.1111/j.1752-4571.2010.00128.x.
- Swain, D.P., and Chouinard, G.A. 2008. Predicted extirpation of the dominant demersal fish in a large marine ecosystem: Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. *Can. J. Fish. Aquat. Sci.* **65**(11): 2315–2319. doi:10.1139/F08-175.
- Swain, D.P., and Foote, C.J. 1999. Stocks and chameleons: the use of phenotypic variation in stock identification. *Fish. Res.* **43**(1–3): 113–128. doi:10.1016/S0165-7836(99)00069-7.
- Swain, D.P., Frank, K.T., and Maillet, G. 2001. Delineating stocks of Atlantic cod (*Gadus morhua*) in the Gulf of St. Lawrence and Cabot Strait areas using vertebral number. *ICES J. Mar. Sci.* **58**(1): 253–269. doi:10.1006/jmsc.2000.1007.
- Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L.P., and Bouvet, J. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Res.* **24**(16): 3189–3194. doi:10.1093/nar/24.16.3189. PMID:8774899.
- Taberlet, P., Waits, L.P., and Luikart, G. 1999. Noninvasive genetic sampling: look before you leap. *Trends Ecol. Evol.* **14**(8): 323–327. doi:10.1016/S0169-5347(99)01637-7. PMID:10407432.
- Tallmon, D.A., Luikart, G., and Beaumont, M.A. 2004. Comparative evaluation of a new effective population size estimator based on approximate bayesian computation. *Genetics*, **167**(2): 977–988. doi:10.1534/genetics.103.026146. PMID:15238546.
- Therkildsen, N.O., Nielsen, E.E., Hüsey, K., Meldrup, D., and Geffen, A.J. 2010. Does DNA extraction affect the physical and chemical composition of historical cod (*Gadus morhua*) otoliths? *ICES J. Mar. Sci.* **67**(6): 1251–1259. doi:10.1534/genetics.103.026146. PMID:15238546.
- Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes*, **4**(3): 535–538. doi:10.1111/j.1471-8286.2004.00684.x.
- Wang, J.L. 2001. A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genet. Res.* **78**(3): 243–257. doi:10.1017/S0016672301005286. PMID:11865714.
- Wang, J.L. 2005. Estimation of effective population sizes from data on genetic markers. *Philos. Trans. R. Soc. B Biol. Sci.* **360**(1459): 1395–1409. doi:10.1098/rstb.2005.1682.
- Wang, J.L., and Whitlock, M.C. 2003. Estimating effective population size and migration rates from genetic samples over space and time. *Genetics*, **163**(1): 429–446. PMID:12586728.
- Waples, R.S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics*, **121**(2): 379–391. PMID:2731727.
- Waples, R.S. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conserv. Genet.* **7**(2): 167–184. doi:10.1007/s10592-005-9100-y.
- Waples, R.S., and Do, C. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Mol. Ecol. Resour.* **8**(4): 753–756. doi:10.1111/j.1755-0998.2007.02061.x.
- Waples, R.S., and Do, C. 2010. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evol. Appl.* **3**(3): 244–262. doi:10.1111/j.1752-4571.2009.00104.x.
- Waples, R.S., and Yokota, M. 2007. Temporal estimates of effective population size in species with overlapping generations. *Genetics*, **175**(1): 219–233. doi:10.1534/genetics.106.065300. PMID:17110487.
- Weir, B.S., and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution*, **38**(6): 1358–1370. doi:10.2307/2408641.
- Westgaard, J.I., and Fevolden, S.E. 2007. Atlantic cod (*Gadus morhua* L.) in inner and outer coastal zones of northern Norway display divergent genetic signature at non-neutral loci. *Fish. Res.* **85**(3): 306–315. doi:10.1016/j.fishres.2007.04.001.
- Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B.C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe, K.A., Stachowicz, J.J., and Watson, R. 2006. Impacts of biodiversity loss on ocean ecosystem services. *Science (Washington, D.C.)*, **314**(5800): 787–790. doi:10.1126/science.1132294. PMID:17082450.

Supplemental materials for Therkildsen et al., CJFAS

Table S1. The number of genotypes obtained for further analysis (*n*), the amplification success, and the genotyping error rates for each locus and sample. See the text for details on computation of error rates.

Loci	<i>Gmo 2</i>	<i>Gmo 8</i>	<i>Gmo 19</i>	<i>Gmo 34</i>	<i>Gmo 35</i>	<i>Gmo 132</i>	<i>Tch 11</i>	<i>Tch 14</i>	<i>Gadm 1</i>	Average
Number of alleles	16	20	24	5	10	8	23	24	7	
Allele size range (bp)	107–153	121–301	120–224	95–111	121–148	111–125	115–215	103–231	168–180	
1928										
<i>n</i> (consensus genotypes)	46	46	40	49	47	47	39	45	45	44.9
Average no. of positive PCRs pr individual	3.3	3.1	3.6	3.3	3.0	2.9	3.6	2.7	2.9	3.1
% of PCRs failed	27.8	25.7	31.1	5.3	19.1	14.1	31.4	21.6	25.5	22.4
% allelic dropout per allele amplified	6.6	7.1	16.0	1.5	9.7	4.1	14.3	6.4	15.1	9.0
% mistaken alleles scored per allele amplified	10.3	13.7	11.5	4.7	4.6	3.0	11.3	7.9	4.2	7.9
% erroneous genotypes per reaction	3.3	2.8	4.2	0	1.4	0	2.8	1.7	0.8	1.9
% of positive PCRs with at least one error	28.7	37.3	50.0	11.8	24.3	11.9	40.8	26.4	26.5	28.6
2008										
<i>n</i>	60	60	60	60	60	60	60	60	60	60
% samples replicated	31.7	31.7	11.7	100.0	30.0	45.0	36.7	25.0	26.7	37.6
% of PCRs failed	0	0	3.3	1.7	1.7	1.7	1.7	1.7	0	1.3
% allelic dropout per allele amplified	2.9	0	0.8	0	2.2	0	0	2.5	0	0.9
% mistaken alleles scored per allele amplified	2.6	0	0	0	0	0	0	0	0	0.3
% erroneous genotypes per reaction	2.6	0	0	0	0	0	0	0	0	0.3
% of positive PCRs with at least one error	5.1	0	1.5	0	2.6	0	0	4.0	0	1.5



Fig. S1. The weighted mean age of spawners by year for females (solid line) and males (dashed line) in the southern Gulf of St. Lawrence cod stock. See the main article for details on the calculation. Based on data from Chouinard et al. (2008) and D. P. Swain (unpublished data).

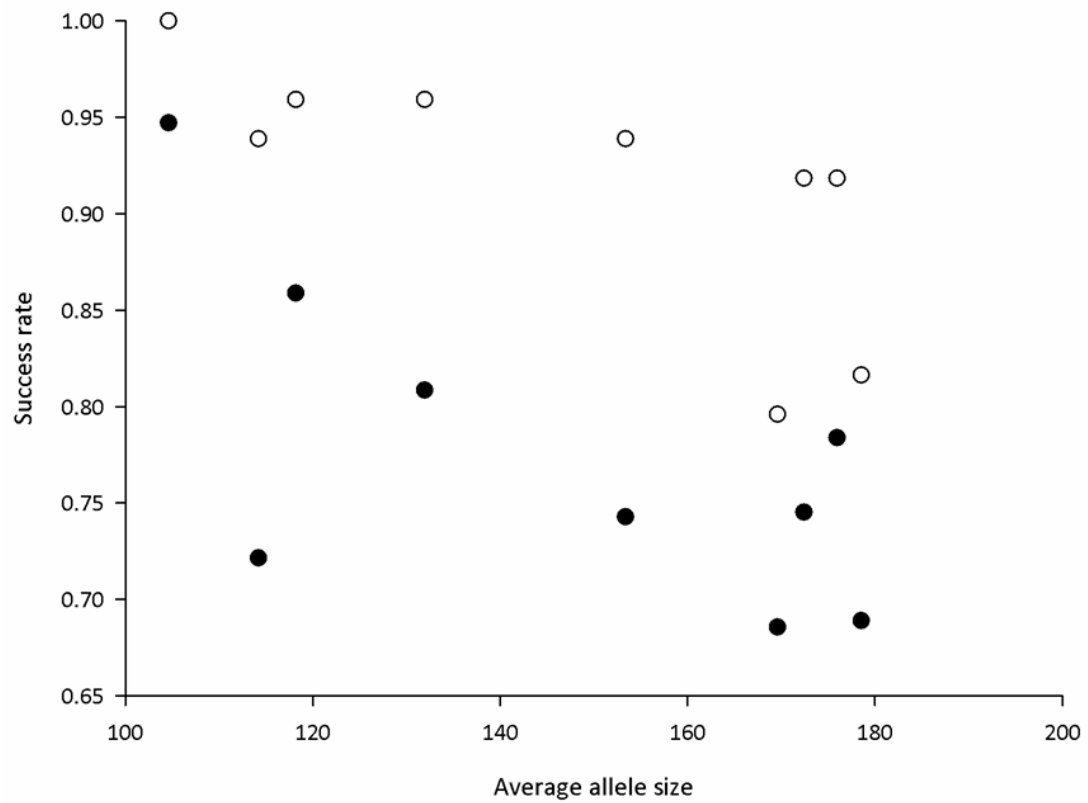


Fig. S2. The amplification success rate (black circles) and the proportion of individuals for which a consensus genotype could be assigned (open circles) in the 1928 sample for each locus in relation to its average allele size.

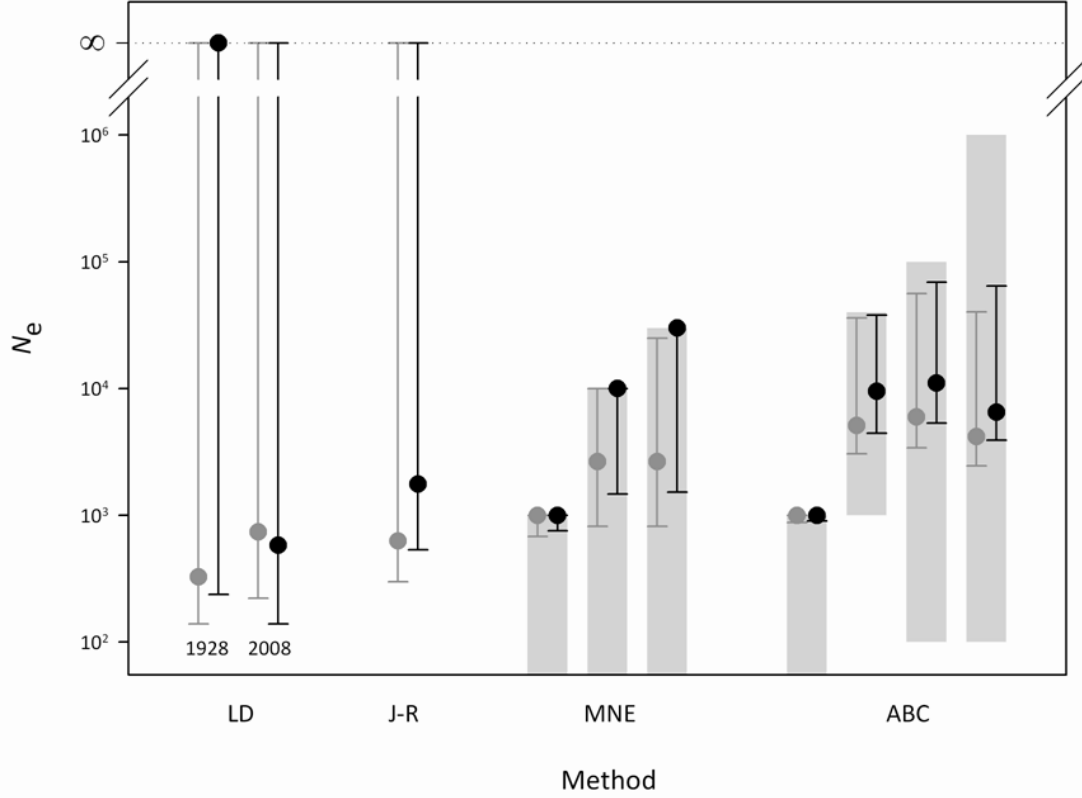


Fig. S3. Point estimates (dots) and precision limits (error bars) of N_e obtained with different estimators on the uncorrected (grey dots and error bars) and consensus data (black dots and error bars). LD is the linkage disequilibrium method applied separately to the 1928 and the 2008 sample. J-R is the Jorde-Ryman temporal method, MNE is the pseudo-likelihood method, and ABC is the method based on approximate Bayesian computation. Error bars represent 95% confidence intervals (2.5% and the 97.5% quartiles of the posterior distribution for the ABC). LD and J-R are moment-based and incorporate no prior. MNE use a uniform prior from 0 to N_{eMAX} and the ABC estimates are based on uniform priors from N_{eMIN} to N_{eMAX} . The priors used in different runs are illustrated by the shaded bars.

CHAPTER 3

Microevolution in time and space: SNP analysis of historical DNA reveals dynamic signatures of selection in Atlantic cod

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ABSTRACT

Little is known about how quickly natural populations adapt to changes in their environment and how temporal and spatial variation in selection pressures interact to shape patterns of genetic diversity. We here address these issues with a series of genome scans in four overfished populations of Atlantic cod (*Gadus morhua*) studied over an 80-year period. Screening of >1000 gene-associated single nucleotide polymorphisms (SNPs) identified 77 loci that showed highly elevated levels of differentiation, likely as an effect of directional selection, in either time, space or both. Temporal allele frequency shifts at certain loci correlated with local temperature variation or with fisheries-induced life history changes. Surprisingly, however, largely non-overlapping sets of loci were temporal outliers in the different populations and outliers from the 1928-1960 period showed almost complete stability during later decades. The contrasting micro-evolutionary trajectories among populations resulted in sequential shifts in spatial outliers, with no locus maintaining elevated spatial differentiation throughout the study period. Simulations coupled with observations of temporally stable spatial structure at neutral loci suggest that population replacement or shifting migration patterns alone could not explain the observed allele frequency variation. Thus, the genetic changes are likely driven by highly dynamic temporally and spatially varying selection, potentially related to fishing pressure. These findings have important implications for our understanding of local adaptation and evolutionary potential in high gene flow organisms and underscore the need to carefully consider all dimensions of biocomplexity for evolutionarily sustainable management.

Keywords: selection, temporal, historical DNA, genome scan, *Gadus morhua*, fisheries-induced evolution

Running title: Microevolution in time and space

INTRODUCTION

In face of accelerated rates of climate change and other growing anthropogenic pressure, it is important to get a better understanding of how quickly natural populations can adapt to altered conditions. The literature contains many examples of rapid evolution in wild populations over contemporary time scales (e.g. Kinnison & Hendry 2001; Palumbi 2001; Stockwell *et al.* 2003; Smith & Bernatchez 2008). However, it is still unclear how widespread such short-term adaptive changes are and under what conditions they occur at rates fast enough to track environmental and human-induced changes (Hendry *et al.* 2008; Hoffmann & Sgrò 2011). Progress in elucidating these important questions has been hampered by the notorious difficulty in demonstrating a genetic basis for apparent local adaptations in natural populations (Gienapp *et al.* 2008; Hoffmann & Willi 2008). There are multiple strategies for disentangling the effects of phenotypic plasticity from genetic differences (recently reviewed by Hoffmann & Sgrò 2011; Hansen *et al.* 2012). Most approaches involve either laboratory experiments such as common garden or reciprocal transplant setups or quantitative genetic analysis that require knowledge of family relationships—both undertakings that can be logistically prohibitive with large, long-lived and highly abundant organisms. For such systems, molecular genetic methods often offer more accessible opportunities for directly observing the underlying genomic signature of selection and adaptive divergence (Nielsen 2005; Storz 2005). Yet, because patterns of genetic diversity integrate effects over millennia, it remains challenging to distinguish historical selection predating colonization of current habitats from ongoing selection. Hence, snapshot observations of the current distribution of genetic variation often tell us little about how stable these patterns are over time or how quickly they may change in response to human activities.

Temporally spaced DNA samples offer a unique opportunity for studying genetic change directly. By comparing the genetic composition of a population before and after a change in environmental conditions, it is possible to track changes in allele frequencies for retrospective ‘real time’ assessment of genetic impacts. Previously, studies using presumably neutral markers have offered important insights about demographic processes including estimates of effective population sizes, loss of diversity, and stability of population structure and migration rates (see reviews by Wandeler (2007), Leonard (2008), and Nielsen and Hansen (2008)). Also, studies targeting specific candidate genes expected to be under selection have begun to elucidate the temporal dynamics of adaptive variation (e.g. Umina *et al.* 2005; Jensen *et al.* 2008; Marsden *et al.* 2012).

Now, with advances in molecular techniques, efforts to study temporal adaptive genetic variation are no longer limited to genes *a priori* expected to be under selection. While neutral evolutionary forces such as drift and migration are expected to leave genome-wide signatures, selection is expected to act only on specific loci and closely linked genomic regions (Cavalli-Sforza 1966; Lewontin & Krakauer 1973). Therefore, comparisons of locus-specific levels of differentiation among large panels of genetic markers, potentially allow for disentangling the effects of neutral processes from the effects of selection. Such “genome scan” approaches are often applied to identify loci affected by selection in space (Luikart *et al.* 2003; Storz 2005; Stinchcombe & Hoekstra 2007), but have only in a few cases been utilized to identify signatures of selection and ongoing adaptation over time in wild populations (notable examples include Hansen *et al.* 2010; Poulsen *et al.* 2011; Bourret *et al.* 2011; Orsini *et al.* 2012), most often due to technical constraints and limited sample availability. Yet, where such challenges can be overcome, simultaneous assessment of both the temporal and spatial scales over which different evolutionary forces are acting, offers extraordinary prospects for gaining more comprehensive insights about the potential for rapid adaptation.

Like many marine fish species, the Atlantic cod (*Gadus morhua*) is characterized by high dispersal ability and a wide distribution with few obvious barriers to migration. Previously, local adaptation was expected to be rare or absent for such species as the homogenizing effects of presumed high

levels of gene flow would swamp the diversifying effects of local selection. However, recent studies based both on genomic signatures of selection on specific loci (e.g. Nielsen *et al.* 2009; Bradbury *et al.* 2010) and common garden experiments (e.g. Marcil *et al.* 2006; Grabowski *et al.* 2009; Harrauld *et al.* 2010) have provided strong evidence in support of adaptive divergence in cod. Signature of divergent selection have been observed even over surprisingly small spatial scales where neutral genetic markers have typically revealed very limited levels of population structure (Hutchings *et al.* 2007; Olsen *et al.* 2008; Poulsen *et al.* 2011).

Recent research has also indicated that cod may possess very high potential for rapid adaptation in response to human impacts. Being one of the historically most important commercial fish species in the North Atlantic, it has been subjected to substantial fishing pressure throughout its range. Theory and modeling work predict that the selection and high mortality imposed by such exploitation can cause large and rapid adaptive changes in the targeted populations (e.g. Ernande *et al.* 2004; Law 2007), a finding also supported by controlled experiments (Conover & Munch 2002; Reznick & Ghalambor 2005). Time series of phenotypic data for many cod populations do indeed demonstrate marked changes in life history traits such as growth and timing of maturation over recent decades (e.g. Trippel 1995; Olsen *et al.* 2005; Swain *et al.* 2007). Statistical analysis has indicated that these changes represent an evolutionary response to fishing (reviewed by Jørgensen *et al.* 2007), although the degree to which such results reflect genetic as opposed to environmentally induced effects remains somewhat controversial (Kuparinen & Merilä 2007; e.g. Hilborn & Minte-Vera 2008). Further, as pointed out by Andersen and Brander (2009), the geographic variation in the affected traits among different populations is often as large as the observed changes over time within single areas (see e.g. Olsen *et al.* 2004; 2005). This raises questions about the role of distributional shifts or altered migration patterns (as opposed to local fisheries selection) as a cause of the observed intra-population trait changes. At the same time, the recent trait changes within single populations may impact the stability of apparent signatures of local adaptation in this high gene flow species.

Capitalizing on recently developed genomic resources and invaluable archived specimen collections, we here address these issues with the—to our knowledge—most extensive tempero-spatial genome scan study on wild populations to date. We focus on a complex of Canadian cod populations that over recent decades have suffered major collapses due to overexploitation and experienced large ecosystem changes in their habitats. By screening temporal and spatial variation in allele frequencies at up to >1000 gene-associated single nucleotide polymorphisms (SNPs), some of which appear to be under selection over larger spatial scales (Nielsen *et al.* 2009; Bradbury *et al.* 2010), we search for loci that show elevated levels of differentiation, indicative of selection, over the past 80 years. Specifically, we ask whether there are (a) any genomic signatures of selection over time within individual populations, (b) if such trends correlate with life history changes or fluctuations in potential drivers of selection, and (c) if parallel temporal patterns are observed across space. We also explore genomic signatures of elevated divergence between populations and assess whether those spatial patterns are stable over time. Finally we evaluate the alternative hypothesis that gene flow alone—rather than ongoing selection—could explain observed patterns of change and discuss the implications of our findings as well as the advantages of conducting simultaneous spatial and temporal analysis for revealing the genetic basis of micro-evolutionary change.

MATERIALS AND METHODS

The study populations and samples

The study was centered on an 80-year time series of samples from a cod population in the southern Gulf of St Lawrence (management division 4T), Canada, but to assess relationships

between temporal and spatial patterns of variation, it also included samples from three nearby management areas (divisions 3NO, 3Ps, 4VsW; Fig. 1). These four populations have exhibited variable demographic trends over the years, but none of them have fully recovered from the severe overexploitation that led to major collapses in virtually all Canadian cod populations in the 1990s. Exemplifying the pattern of apparent adaptive divergence in both time and space described above, common garden experiments have indicated clear, genetically based functional differences among these particular populations for a number of traits (Marcil *et al.* 2006; Hutchings *et al.* 2007) and more or less parallel reductions in growth rate and/or timing of maturation have also been observed within all of them over recent decades, likely reflecting fisheries-induced evolutionary change (Hutchings 2005; Olsen *et al.* 2005; Swain *et al.* 2007; Swain 2011).

Contemporary samples of gill tissue were collected from all populations on research cruises during 2008-2010. Historical samples consisted of archived otoliths that had been stored individually in paper envelopes at room temperature since collection. The oldest otoliths from 1928 were obtained from DTU Aqua, Denmark where they had originally been acquired for a study of cod “races” (Schmidt 1930). All other otoliths were obtained from the collections at Fisheries and Oceans Canada. Sets of at least 30 otoliths were selected for single years between 1928-2008 based on availability (see Table 1 for final sample sizes and years). All individuals, with the possible exception of the 1928 sample, for which the sampling time is unknown, were collected during the spawning season and were of reproductive age.

DNA extraction and genotyping

DNA was extracted with Omega EZNA Tissue DNA kits (Omega Bio-Tek, USA) following the manufacturer’s instructions for fresh tissue and the procedure described by Therkildsen *et al.* (2010a) for otoliths. To pre-screen DNA extracts, we amplified four highly polymorphic microsatellites (mean number of alleles 19) in all samples using a PCR multiplex kit (Qiagen, Germany) and analyzed the fragments on an ABI 3130 Genetic Analyzer (Applied Biosystems, USA). We removed individuals that showed evidence of cross-sample contamination (amplification of >2 alleles for any locus) or that failed to produce reliable amplification within 2-3 attempts. For the historical samples, both DNA extraction and PCR preparation were conducted in an ancient DNA laboratory or a separate facility where no contemporary fish samples had been processed.

Samples that passed the pre-screening were genotyped for a set of gene-associated SNPs, primarily developed by the Canadian Cod Genomics and Broodstock Development Project (Hubert *et al.* 2010; Bowman *et al.* 2011). We used an initial panel of 1536 SNPs (see Nielsen *et al.* 2012) and to position as many SNPs as possible on the cod linkage map (Hubert *et al.* 2010) and obtain annotation information, we mapped the 120 bp flanking sequence of each SNP to the cod genome sequence (see Supporting information Note 1). In a trade-off between the number of samples and the number of SNPs to analyze, we applied a two-step approach: Initially, we only scanned the end points of the longest available time span, i.e. the 1928 and the contemporary sample from 4T, with the full 1536 SNP panel. Since the majority of SNPs showed no temporal variation in allele frequencies among these samples (see below), all other samples were analyzed with only a subset of SNPs, including the 50 loci that showed the largest temporal changes in the initial scan, 29 candidate genes for life history traits (Hemmer-Hansen *et al.* 2011), 23 loci that had been shown to be under selection in this species on broader geographical scales (Nielsen *et al.* 2009; Bradbury *et al.* 2010), and a random selection of the remaining loci for a total of 182 SNPs.

All SNP genotyping was performed by the Roslin Institute at the University of Edinburgh, Scotland, using the Illumina GoldenGate platform (Fan *et al.* 2006) following the manufacturer’s protocol. This array-based technology relies on hybridization of short (<60 bp) locus- and allele-specific probes to the template DNA and should therefore be well suited for historical DNA that typically is

fragmented. To minimize the risk of cross-sample contamination, historical and contemporary samples were kept separate during all steps. The SNP data were visualized and analyzed with the GenomeStudio Data Analysis Software package (Illumina Inc.).

Data quality control

To ensure reliability of the SNP data despite the degraded nature of DNA from historical samples, we implemented several data control procedures and applied a conservative quality filtering. First, genotypes were called based on manual editing of all SNP cluster positions. Second, 29 DNA extracts were re-genotyped in independent assays to assess the reproducibility of results, and we excluded SNPs yielding <0.7 reproducibility rate between the genotypes in replicate samples. Third, we only included data points with a GenCall score of >0.4 (a data quality metric; Illumina recommends a standard threshold of 0.25 (Illumina 2008)) and excluded SNPs and samples that following this strict filtering yielded call rates (percentage of successful genotype calls) < 0.5.

We computed expected (H_e) and observed heterozygosity (H_{obs}) and tested for Hardy-Weinberg equilibrium (HWE) in all samples using 10^5 permutations with the Monte Carlo procedure implemented in the R-package adegenet (Jombart 2008). The degree of linkage disequilibrium (LD) between all pairs of loci within each sample was evaluated with the genetics package in R (Warnes 2003). Here and where appropriate throughout the analysis, we corrected for multiple testing by computing the expected false discovery rate (FDR), or q-value, for each test based on the distribution of p-values using the R-package qvalue (Storey & Tibshirani 2003). We considered tests significant when the FDR was <5% ($q < 0.05$).

Temporal outlier detection

Identifying loci that show divergent patterns of differentiation in temporally spaced samples is conceptually similar to searching for outliers in samples collected from different spatial populations. We therefore applied a modified version of the commonly used approach of Beaumont and Nichols (1996) to detect temporal outlier loci within each of the four populations. Based on the premise that selection should affect only certain parts of the genome whereas neutral evolutionary forces should cause genome-wide effects, this method compares the observed locus-specific differentiation as a function of heterozygosity to a null distribution generated through simulations.

Here, we adapted the original outlier detection method to fit our scenario by generating the expected null distribution of genetic change through simulations of drift within a single isolated population rather than drift-migration equilibrium between multiple demes. Our null model was based on multi-generational sampling of a Wright-Fisher population that for each scenario was parameterized with the number of generations between sampling points, the harmonic mean of sample sizes, and the effective population size (N_e) estimated for each population with the software MLNE (Wang 2001 see supplementary material for details). Following the procedure in Beaumont and Nichols (1996), the simulated distribution was then used to identify outlier loci that varied more over time than expected due to drift and sampling error (see Supporting information Note 2).

We conducted separate temporal genome scans for the initial SNP panel genotyped in the 4T 1928 and 2008 samples and for the subsequent samples from each of the four populations, every time basing the null distribution on 10^5 simulated data points. All simulations and computations were completed with custom R-scripts (available upon request).

Spatial outlier detection

In addition to testing for temporal outliers within each population, we also looked for spatial outliers among the populations at three periods in time: the 1960s, the 1980s-1990s (here we had no sample from 4VsW), and among contemporary samples. For this analysis, we applied the standard *fdist2* model (Beaumont & Nichols 1996) as implemented in the software *Lositan* (Antao *et al.* 2008). This method is based on the same approach as the temporal genome scans, but here the neutral expectations are generated through coalescent simulation under an island model. We used 10^5 iterations, the infinite alleles mutation model and assumed 30 demes (varying input parameters did not change results). For extra validation, we compared our results to outputs from a different commonly applied spatial genome scan method, the Bayesian approach of Beaumont and Balding (2004) as implemented in the program *BayeScan* (Foll & Gaggiotti 2008).

Correlation to environmental variation

The moderate number of samples in this study precludes rigorous statistical testing of how allele frequency shifts may correlate with environmental or phenotypic variables. However, to investigate what factors may be associated with temporal shifts within the 4T population, which was sampled at seven time points, we computed Pearson's correlation coefficients (r) between allele frequencies at temporal outlier loci and data on a suite of environmental and demographic factors for the sampled years. The factors included fishing mortality, temperature, biomass, and indices of growth rate and length at maturation (see Table S1, Supporting information, for a full list of variables and data sources). Based on the obtained coefficients, we compared the relative degree of correlation between outlier loci and explanatory variables and further examined the strongest observed patterns.

Differentiation among samples

We used a hierarchical AMOVA with time points nested within populations to assess how the overall genetic variation was distributed in space and time. This was done in *Arlequin* vers. 3.5 (Excoffier & Lischer 2010) and the significance of contributions from the different levels was tested with 10000 permutations. Pairwise F_{ST} between all samples was computed with the *Fstat* function from the *Geneland* package in R (Guillot *et al.* 2005), and we tested for pairwise differences in allele frequencies among all samples using Chi-square tests, as implemented in the software *Chifish* (Ryman 2006).

To obtain estimates reflecting signatures of neutral evolutionary forces only, we repeated all these analyses on a reduced set of loci ($n=101$), excluding all spatial and temporal outlier loci (see below). We also used the program *Powsim* 4.0 v4.1 (Ryman & Palm 2006) to evaluate our power to detect genetic heterogeneity in the different comparisons.

To visualize the patterns of sample differentiation, we applied principal coordinates analysis (PCoA) to the pairwise F_{ST} matrices using the *ade4* package in R (Jombart 2008). The weak differentiation between populations (see Results) provided insufficient power to apply genetic clustering methods, assignment tests or admixture proportion estimation to further elucidate the neutral population structure and its stability through time. We did try to apply these methods, but without getting meaningful results, as expected when differentiation is very low (see Waples & Gaggiotti 2006).

Contemporary migration

The low levels of differentiation also precluded estimation of contemporary migration rates (Wilson & Rannala 2003; Faubet *et al.* 2007). However, to evaluate if migration rather than selection could explain our observations, we constructed simulations to elucidate how much migration would be needed if gene flow alone should have caused the observed temporal variation at outlier loci. Assuming that the 1960s samples reflected baseline allele frequencies for the four populations, we simulated various levels of exchange (migration rate m ranging from 0-1) between populations over the sampling period and analyzed these simulated data with our temporal genome scan model (see Supporting information Notes 2 and 3). For each population, we evaluated the number of significant temporal outliers under different combinations of m , local N_e and source population of migrants as well as how many of these outliers were identical to the temporal outliers in the observed data.

RESULTS

Data quality and genetic diversity

A total of 508 samples could be used for analysis, while 137 were discarded due to poor amplification, contamination or low quality SNP genotyping. For the initial scan, 1047 SNPs were successfully genotyped, passed the quality criteria and were polymorphic in at least one sample. For the follow-up panel, 160 SNPs (of 182) could be used for analysis. The error rate among replicate samples was <5% for all historical samples and <1% for contemporary samples and the mean SNP call rate was >90% for all included loci (except from the 1928 sample where the mean call rate was 0.76).

On average 85% of the loci were polymorphic in each sample (Table 1). The average H_e within samples was 0.26 and there was no clear relationship between H_e or the proportion of polymorphic loci and the sampling year (Table 1). In the 1047-SNP data set, 31 tests involving 25 SNPs showed significant departures from HWE proportions after FDR correction and 6 loci showing departure in >1 sample were excluded from analysis. In the 160-loci set, we observed only a single significant departure from HWE after FDR control among samples.

In the 1047-SNP data, between 1.7 and 2.7% of pairwise tests for the LD were significant ($q < 0.05$). In the 160-SNP data, between 2.1 and 5.8% of pairwise tests for LD between loci within each sample were significant ($q < 0.05$). From this panel, almost all SNPs that showed significant LD in multiple samples originated from one of three clusters on different linkage groups and were outliers either in space or time (see below).

Temporal outliers

The generation lengths for the four populations were estimated to be 6-8 years so our sampling covered 6-12 generations (Table S2, Supporting information). The temporal variation in allele frequencies over this period indicated that the N_e was >30 000 (the maximum estimation capability of the applied method) in all populations and the lower 95% confidence limit was always >500 (Table S2, Supporting information).

In the initial comparison between 1928 and 2008 in 4T, the temporal differentiation at 50 of the 1047 loci exceeded the 95% confidence limit for neutral expectations, but only 10 remained significant ($q < 0.05$) after FDR correction (Fig. 2a). Nine of these (and in total half of the 50 outliers) were successfully genotyped in the additional samples. Surprisingly, the vast majority of the initial

outlier loci did not show increased temporal differentiation among the second set of samples. Although we observed allele frequency changes of >50% between 1928 and 2008 in 4T, none of these loci showed outlier patterns in the intermediate time period from 1960-2002 within 4T (Fig. 2b, Table 2). Examination of the temporal data showed that the changes had primarily occurred between 1928 and 1960 and that allele frequencies at these loci had remained stable from 1960 to 2008 (Fig. 3a). Similar patterns of stability were observed in the other populations, although one locus from the initial comparison was also a significant temporal outlier in 3NO (Fig. 3b-d).

Considering only the samples collected between the 1960s and 2000s, the genome scans identified an entirely different set of loci as outliers. Between 7 and 14 of the 160 loci genotyped here fell above the 95% confidence envelope for null expectations in the different populations, but only in 3NO and 4T were >1 locus significant after FDR correction (Fig. 2, Table 2). In 3Ps, a subset of the significant 3NO outliers also showed increased differentiation, but interestingly, there was basically no overlap between the outliers of 3NO and 4T (Table 2). 4VsW showed an intermediate pattern where subsets of both 3NO and 4T outliers as well as an additional group of loci showed increased differentiation (Table 2). Examination of outlier allele frequencies revealed that the 3NO outliers in fact also showed large differentiation within 4T, but mostly between 1928 and 1960 (and therefore were not detected in the 1960-2002 genome scan). The 4T outliers, however, remained stable in 3NO and 3Ps throughout the study period, indicating clear non-parallel trajectories for these loci among the populations (Fig. S1, Supporting information).

Spatial outliers

The Lositan analysis indicated that the differentiation at 7-15 loci exceeded the 95% confidence limit on neutral expectations in the spatial comparisons for different time periods (Fig 4, Table 2). BayeScan generally identified fewer outliers (in some comparisons none at all), but never loci that were not identified by Lositan and it was qualitatively consistent in identifying the most differentiated loci (Fig. S2, Supporting information).

Comparison of the three snapshots in time revealed a marked sequential shift in the loci exhibiting spatial divergence, with no overlap between the 1960s and the contemporary spatial outliers, while the spatial outliers from the 1980s-90s comparison showed overlap both with the time period before and after (Table 2). No locus remained a spatial outlier at all time periods. Removing the 4VsW sample from the spatial comparisons resulted in much fewer and some different outliers (Fig. S3, Supporting information), suggesting that this population is driving much of the overall outlier pattern observed. However, this population was not sampled in the 1980s-1990s period that overall showed the highest number of outliers, so the locus-specific patterns of spatial divergence are clearly highly dynamic.

The dynamic pattern of spatial divergence is further supported by the match between temporal and spatial outliers. The spatial outliers in the 1960s were a subset of the loci that were temporal outliers within 3NO (and to a lesser degree 3Ps and 4VsW). Since these loci were no longer spatial outliers at later time points, the temporal changes have homogenized allele frequencies among populations. The contemporary spatial outliers, on the other hand, were for a large part the same loci that were temporal outliers in 4T (and to a lesser degree 4VsW). Since these loci were not spatial outliers in the 1960s, the temporal changes of outlier loci in 4T and 4VsW caused greater divergence among populations over time.

In total, 33 loci were outliers either in space, time or both (in the post-1928 data). These were spread over at least 11 linkage groups and generally displayed low and non-significant levels of LD between them (Fig. S4, Supporting information). However, 13 of the outliers clustered into two high LD groups, each spanning 10-14 cM and mapping to 5-6 different scaffolds that combined cover

>2Mb in the Ensembl cod genome assembly (www.ensembl.org; release 65, Dec 2011). Most outlier SNPs were located in the 3' UTR of gene models (Table S3, Supporting information).

Correlation to environmental variation

An index of ambient temperature for cod during the feeding season showed the best temporal correlation with allele frequencies at the temporal outlier loci in 4T ($r > 0.8$ for most loci), indicating a co-varying temporal pattern (Fig. 5a). A similar correlation was also evident for two other outlier loci (Fig. S5, Supporting information). Within 4T, a set of loci initially identified as temporal outliers in 3NO also showed a strong correlation ($r > 0.9$) to temporal shifts in estimated probabilistic maturation reaction norm midpoints (Fig. 5b), a life history change expected to reflect an evolutionary response to fishing (Swain 2011). These loci also showed correlation with the temporal trend in total mortality rate ($r > 0.8$; Fig. S5, Supporting information).

Differentiation among samples

The AMOVA revealed that for certain loci, differences between populations or between time points within populations accounted for up to 12% of the total variation (Fig. S6, Supporting information). For the total set of post-1928 outlier loci ($n=33$), temporal and spatial dimensions explained about equal amounts of variation (on average 2.7% of the total variation was found between time points within populations and 2.1% between populations, both levels being highly significant ($p < 0.0001$)).

For the non-outlier loci that presumably reflect neutral population structure, a much smaller proportion of the variance was partitioned among the hierarchical levels. There was low but significant spatial variation ($p=0.0064$), but no significant variation among time points within populations ($p=0.87$; Table 3). These results of very weak but temporally stable spatial structure were corroborated by the pairwise tests for differences in allele frequencies. With all loci, pairwise F_{st} ranged from -0.007 to 0.086 and 31 tests were significant after FDR correction including comparisons both within and among populations. When only considering the “neutral” set of loci, no comparisons were significant after FDR correction (Table S6, Supporting information). The mean pairwise F_{st} among samples for these loci was 0.0015 and simulations indicated that with the applied panel of markers and sample sizes, we would only have a 14% chance of detecting significant differentiation at this level (results not shown). However, by pooling the time points within areas, we should have >75% chance of detecting differentiation among populations, and we did indeed find significant differences in allele frequencies ($p < 0.04$) between all combinations except those involving 3Ps, which could not be differentiated from 3NO and 4T. Consistent with the barely detectable—yet significant—level of differentiation, the PCoA plots did not show clear separation of the samples. However, there is a vague tendency for samples from the same populations to cluster together along the primary axes of variation although there are differences between sampling years (Fig. S7, Supporting information).

Contemporary migration

Simulations indicated that in general, migration rates of 0.2-0.3 would be required to generate a similar number of significant temporal outliers as were observed in the different populations (Fig. S8, Supporting information). For 3NO, somewhat higher migration rates of >0.6 could have driven allele frequency changes at the specific loci that were observed as significant temporal outliers in the actual data (Fig. S9, Supporting information). However, this was only if migrants originated from 4VsW—the population that 3NO was the most differentiated from at neutral markers (Tables

S5 and S6, Supporting information). In 4T, even complete replacement by any of the other populations could not account for the frequency shifts at the observed outlier loci (on average <1 of these loci were identified as outliers in the migration simulations regardless of N_e and m values assumed; Fig. S9, Supporting information).

DISCUSSION

This study revealed highly heterogeneous patterns of differentiation among SNPs from different regions of the cod genome, with certain gene-linked SNPs showing substantially elevated divergence in either time, space, or both. Over the short time scale and small geographical area considered, we observed markedly different microevolutionary trajectories within four adjacent populations and found a temporal shift in which loci showed increased differentiation during different time periods. Because the majority of genomic locations showed almost complete stability in allele frequencies, the significantly higher differentiation at specific loci likely reflects effects of selection either directly at these loci or at closely linked sites.

Patterns of selection

If selection indeed is the primary driver behind the observed allele frequency shifts at outlier loci, then this study indicates highly variable local selection pressures that both target different regions of the genome and work at varying strengths and directions depending on the area and time period considered. Most notably, the majority of temporal outliers in the 1928-2008 comparison in 4T showed little change between 1960 and 2008, a period during which large allele frequency shifts were observed at a completely different set of loci. This indicates that different parts of the genome may have been under selection early and late in the study period. Similarly, a subset of the 3NO temporal outlier loci were not outliers in 4T between 1960 and 2002, but exhibited a slight clinal pattern apparent through comparison with the 1928 sample. This may suggest that these loci were also under selection in 4T, but at an earlier time than in 3NO.

Varying power to detect outliers caused by the non-symmetric sampling pattern (imposed by limited sample availability) probably explains a portion of the variation in which and how many loci were detected as statistically significant temporal outliers in the different populations. For example, the smallest numbers of significant temporal outliers were found in 3Ps and 4VsW—the populations for which we had the fewest samples. However, qualitative comparison makes it clear that the largest changes were found at different loci in the different populations and that patterns of allele frequency change at particular loci were often non-parallel.

A number of outlier loci showed gradual and directional changes over time, but many also showed more fluctuating, apparently ephemeral patterns. Such unstable patterns of selection are in line with the combined inference from numerous temporally replicated studies on phenotypic selection in natural populations, which indicate that strong temporal variation in both the direction and strength of selection is the norm rather than the exception over short time scales (recently reviewed by Siepielski *et al.* 2009; Bell 2010; Kingsolver & Diamond 2011). Although some of the patterns reported in these studies may be caused by sampling error (Morrissey & Hadfield 2012), short-term fluctuations in selection pressures may be particularly common in a highly dynamic and stochastic environment like the ocean, so they would explain our observed patterns well.

Drivers of selection

Our exploratory correlation analysis showed that allele frequencies at temporal outliers in 4T seemed to track variation in ambient fall temperature, indicating that this variable could reflect a possible driver of selection. Although based on relatively few data points, this relationship is supported by a previous finding of strong temperature-associated clines in allele frequencies at these particular loci over large spatial scales (Bradbury *et al.* 2010). The lack of correlation with other tested temperature variables could suggest that the index of ambient temperature, which summarizes the local temperature measurements in areas where cod have been most abundant for a particular year, is a more biologically relevant variable than coarser scale measurements. Genotype-environment correlation analyses are always limited by the difficulty in identifying and obtaining data on the dimension of environmental variation that is of importance to the organism (Hansen *et al.* 2012). This is particularly challenging in temporal studies where cumulative effects and unknown time lags often will be important.

These challenges may explain why we did not observe direct correlations between any outliers and the instantaneous fishing pressure among 4T outliers (although some loci seemed to correlate loosely with the total mortality rate). The contrasting trajectories for allele frequency shifts at outlier loci in the four populations could be interpreted as evidence for fisheries not being a dominant driver of selection overall as we might have expected parallel signatures of selection across these highly exploited populations. However, although all four populations have been fished heavily over the study period, small-scale differences in exploitation patterns and intensity may have mediated variations in the specific selective response, so our results do not rule out fishing as an important driver.

Notably, in 4T, allele frequency changes at a set of linked loci strongly correlated with temporal shifts in probabilistic maturation reaction norms that are expected to reflect an evolutionary response to fishing (Swain 2011). Also, the pattern of variation among the 4T outliers in linkage group 12 corresponds to some extent with temporal shifts in the size selectivity imposed by fishing (Sinclair *et al.* 2002; Swain *et al.* 2007). While these correlations do not establish functional causality, they indicate that some of the observed patterns of selection could be related to fisheries-induced phenotypic changes in life history traits. The correlation with observed phenotypic changes is less clear-cut for some of the other populations with fewer samples, but our initial synoptic findings here are consistent with both temperature- and fisheries-induced selection pressures acting over the study period.

Targets of selection

The strength of genome scan approaches, as applied here, is that they do not require the target of selection to be known *a priori* and that observed patterns are intrinsically genetic in contrast to quantitative trait changes, which can be partly or fully environmentally induced. The drawback of the genome scan approach is that, especially for non-model organisms, it can be difficult to establish the phenotypic significance and fitness effects of interesting genetic polymorphisms. A first step is to identify the exact targets of selection. Since most of the SNPs studied here were located in 3' UTR regions of various genes and the few that were located in coding sequence were synonymous polymorphisms, we are unlikely to have identified the exact causative mutations (although the loci may have regulatory roles). We have, however, narrowed in on important candidate genes and we noted that many outlier loci were found in regions with genes related to metabolism (see supplementary material), indicating a possible relationship with life history traits. These findings constitute valuable starting points for future more detailed efforts to identify specific targets of selection.

The role of gene flow

Conceivably, dynamic dispersal rather than spatially and temporally varying selection pressures acting within separate populations over the study period could explain the large allele frequency shifts observed. Generally, migration is expected to have genome-wide effects (Cavalli-Sforza 1966; Lewontin & Krakauer 1973), but it will only leave observable signatures at loci where allele frequencies differ between demes. Because allele frequency differences at non-outlier loci were so small among the four populations, the elevated temporal differentiation at specific loci could in principle have been caused by increased migration from a deme (or number of demes) that differed in allele frequencies only at those particular loci. Such locus-specific differentiation between demes would likely have arisen due to selection, but would primarily reflect effects accumulated in the past, potentially over long time scales. To better understand mechanistically how microevolution operates in this system, it is critical to distinguish between pre-adapted demes moving in and out of the sampling sites and ongoing changes in which genotypes survive and reproduce within specific locations.

While the low level of differentiation at neutral loci could indicate high gene flow, it could also reflect large N_e 's and recent divergence that have limited the accumulated effects of drift. Here, our results suggested a large N_e in all populations, consistent with a previous temporal study of the 4T population using microsatellites (Therkildsen *et al.* 2010b). Because the evolutionary consequences of gene flow scale with the absolute number of effective migrants, $N_e m$ (Wright 1931), the finding of large N_e 's imply that even extremely small migration rates could homogenize allele frequencies at neutral loci. However, our simulations suggest that extreme migration rates of at least 0.2 - 0.3 would be needed to cause the strong shifts in allele frequencies we observed within populations. Such high migration rates are beyond levels normally associated with separate populations (Waples & Gaggiotti 2006) and are at odds with ecological data that suggest substantial reproductive isolation of the studied populations. Consistent phenotypic differences have been demonstrated (Swain *et al.* 2001), and despite extensive seasonal migrations, studies based on both traditional tags and otolith microchemistry have indicated that in 4T, almost all fish return to spawn with their local population (Campana *et al.* 2000; Robichaud & Rose 2004), and this is also true for 4VsW (Robichaud & Rose 2004). Tagging results are more mixed for the two other populations (Robichaud & Rose 2004), but much of the reported population mixing occurs outside the spawning season and samples from these populations were specifically selected from locations that are expected to be minimally affected by migration. Clearly, tagging studies on adults provide no information about dispersal during the larval stage. However, although the dispersal potential of this life stage is high in cod (Helbig *et al.* 1992; Pepin & Helbig 1997), several recent studies have demonstrated surprisingly high retention rates within a few kilometers depending on local oceanographic conditions (Knutsen *et al.* 2007; Bradbury *et al.* 2008). Predominant currents out of the Gulf of St Lawrence make external larval input into the 4T population unlikely and modeling predicts a high degree of local retention, although a small portion of its larvae may drift to the other management areas (Chassé 2003).

However, if migration rates from larval drift should amount to >0.2 and N_e 's are at the very least 500 and probably much higher (Table S2, Supporting information), then the $N_e m$ would be $\gg 100$, a number much greater than what is typically considered a threshold for genetic separation of populations (Waples & Gaggiotti 2006). Such a high $N_e m$ seems highly inconsistent with the temporally stable spatial genetic structure that, although weak, was observed here and that has also been demonstrated previously with microsatellites among several of these populations (Ruzzante *et al.* 1996; 2000; 2001; Beacham 2002).

In addition, although the increased differentiation at the specific outlier loci in 3NO could have been caused by very high levels of migration from a mix of the other populations, this was not the case for 4T where none of the other populations could have served as a source for the divergent allele

frequencies at outlier loci. We cannot completely exclude that some unsampled “ghost” population has contributed such migrants, but given that the Gulf of St Lawrence is a semi-enclosed basin that hosts a special set of oceanographic conditions, potential sources are difficult to imagine. Thus, although gene flow likely plays a role in this system, it is highly implausible that it has been the main driver behind the observed patterns at outlier loci in this study.

Other alternative explanations

A number of additional factors could potentially have affected our results. First, genotyping errors, either random or related to variable levels of DNA degradation in the historical samples, could cause false impressions of large allele frequency differences. However, while suboptimal DNA quality inevitably has caused a few inaccuracies in our dataset, the stringent quality control measures implemented and the resulting high degree of reproducibility of genotyping clearly indicates that this effect has been minor. There is no relationship between sample age and observed genetic diversity (Table 1), and outlier loci do not exhibit significantly elevated error rates (Fig. S10, Supporting information). The strongest verification of data reliability, however, comes from the highly consistent patterns of strong LD among outlier loci across all samples including the 4T 1928 (Fig. 5, Fig. S4, Supporting information).

Second, violated model assumptions or general methodological limitations may have resulted in false positives among the identified outliers. However, the differentiation at the majority of outlier loci was substantially elevated compared to the rest of the loci, i.e. not just at a tail of a continuous distribution (Fig. 2 and 4). Hence a slight change in the cut-off for significance that may change the status of a few weak outliers would not affect conclusions about these highly divergent loci. Additionally, previous studies have demonstrated that many of the outlier loci identified here also show elevated differentiation among cod populations across much larger spatial scales (Nielsen *et al.* 2009; Bradbury *et al.* 2010), supporting their affiliation with selection.

Third, when temporal variation exceeds spatial differences in genetic studies of marine organisms, the pattern is often attributed to random genetic patchiness caused by extremely high variance in reproductive success, termed “sweepstakes recruitment” (Hedgecock 1994; Hedgecock & Pudovkin 2011). With successive waves of offspring originating from relatively few parents that happened to match favorable conditions during spawning, the increased drift within each cohort can leave ephemeral signatures of differentiation. However, this process would create temporal heterogeneity across the entire genome, not only at specific loci, so it is unlikely to have caused the patterns we observe. Hence, in the absence of convincing alternative explanations, it seems highly likely that temporally and spatially varying selection has played an important role in shaping the large locus-specific allele frequency changes observed in this study.

Implications and future research

The finding of highly dynamic signatures of selection in western Atlantic cod populations raises the question of whether our observations reflect a general pattern of microevolution in marine fish. Only very few other studies have so far examined temporal variation in genetic markers under selection. Focusing on just a single locus, the well-studied Pan-I, Nielsen *et al.* (2007) found temporal stability in four cod populations over decadal time scales, whereas two studies have presented evidence of potentially fisheries-induced temporal changes in allele frequencies for this locus in Icelandic cod (Árnason *et al.* 2009; Jakobsdóttir *et al.* 2011). The latter findings are in line with the results here where Pan-I also was appeared to be under temporal selection. We showed,

however, that it along with 5 other outliers was part of a tight LD group spanning >2Mb of the genome, indicating that this locus may just be a marker for the region, not the target of selection.

A previous temporal genome scan in cod reported stability of allele frequencies over a 24-year period at the Faroe Bank (Nielsen *et al.* 2009), but a different study showed marked temporal shifts in allele frequencies at several loci in other cod populations in the Northeast Atlantic (Poulsen *et al.* 2011). Thus there is evidence that ongoing selection over short time scales may be a widespread phenomenon at least for cod but also likely in other marine fish populations; future research using retrospective genetic analysis (Nielsen & Bekkevold 2012) are needed to generate further insights into its extent.

Despite that direct phenotypic and fitness effects of observed outlier loci remain elusive, the findings here add an important new perspective to recent studies presenting evidence for local adaptation in marine fish either through elevated divergence at specific loci (e.g. Nielsen *et al.* 2009; Bradbury *et al.* 2010) or common garden experiments (e.g. Marcil *et al.* 2006; Grabowski *et al.* 2009; Harrauld *et al.* 2010). Our study illustrates that where temporal stability of genetic divergence has not been demonstrated, it can clearly not be assumed *a priori*. If environmental conditions are highly dynamic, local adaptation may also not necessarily imply static differences between populations, but can reflect ongoing changes. Findings of greater temporal than spatial variation at certain loci may be limited to studies that, like here, focus on a small geographical area over which environmental conditions are relatively similar. However, future studies over larger spatial scales should provide additional insights about the interactions between temporal and spatial variation in selection pressures. In any case, the large changes observed over just a few generations—in some cases correlating with environmental or life history trait variation—suggest that cod populations can respond rapidly to changes in selection pressures and therefore may be able to quickly adapt to human-induced modifications of their environment.

A better understanding of temporal and spatial scales of adaptation will be crucial both for our fundamental understanding of microevolution in high gene flow organisms and for conservation and fisheries management. The importance of intraspecific diversity in fitness-related traits for ensuring stability and persistence of species and fisheries yields is increasingly being recognized (Hilborn *et al.* 2003; Hutchinson 2008). Adaptive divergence between populations or between subunits of metapopulations can generate ‘portfolio effects’ that dampen overall fluctuations in abundance because if various population components are adapted to different conditions, they may exhibit independent and complimentary reactions to perturbations (Hilborn *et al.* 2003; Schindler *et al.* 2010). Our results here demonstrate that it is critical to consider not only the spatial but also the temporal dimension of this biocomplexity and to evaluate how human activities affect the overall system resilience.

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LITERATURE CITED

- Andersen KH, Brander K (2009) Expected rate of fisheries-induced evolution is slow. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 11657–11660.
- Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: A workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics*, **9**, 323.
- Árnason E, Hernandez UB, Kristinsson K (2009) Intense habitat-specific fisheries-induced selection at the molecular *Pan* I locus predicts imminent collapse of a major cod fishery. *PLoS ONE*, **4**, e5529.
- Beacham T (2002) Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation. *ICES Journal of Marine Science*, **59**, 650–665.
- Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology*, **13**, 969–980.
- Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **263**, 1619–1626.
- Bell G (2010) Fluctuating selection: the perpetual renewal of adaptation in variable environments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 87–97.
- Bourret V, O'Reilly PT, Carr JW, Berg PR, Bernatchez L (2011) Temporal change in genetic integrity suggests loss of local adaptation in a wild Atlantic salmon (*Salmo salar*) population following introgression by farmed escapees. *Heredity*, **106**, 500–510.
- Bowman S, Hubert S, Higgins B *et al.* (2011) An integrated approach to gene discovery and marker development in Atlantic cod (*Gadus morhua*). *Marine Biotechnology*, **13**, 242–255.
- Bradbury IR, Hubert S, Higgins B *et al.* (2010) Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **277**, 3725–3734.
- Bradbury IR, Laurel BJ, Robichaud D *et al.* (2008) Discrete spatial dynamics in a marine broadcast spawner: Re-evaluating scales of connectivity and habitat associations in Atlantic cod (*Gadus morhua* L.) in coastal Newfoundland. *Fisheries Research*, **91**, 299–309.
- Campana SE, Chouinard GA, Hanson JM, Frechet A, Bratley J (2000) Otolith elemental fingerprints as biological tracers of fish stocks. *Fisheries Research*, **46**, 343–357.
- Cavalli-Sforza L (1966) Population structure and human evolution. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **164**, 362–379.
- Chassé J (2003) Modeling of cod eggs and larvae drift, growth and survival in the Gulf of St. Lawrence, Canada. *ICES CM*, **2003/O:02**, 6 pp.
- Conover DO, Munch SB (2002) Sustaining fisheries yields over evolutionary time scales. *Science*, **297**, 94–96.
- Ernande B, Dieckmann U, Heino M (2004) Adaptive changes in harvested populations: plasticity and evolution of age and size at maturation. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **271**, 415–423.
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.
- Fan J-B, Gunderson KL, Bibikova M *et al.* (2006) Illumina universal bead arrays. *Methods in enzymology*, **410**, 57–73.
- Faubet P, Waples RS, Gaggiotti OE (2007) Evaluating the performance of a multilocus Bayesian method for the estimation of migration rates. *Molecular Ecology*, **16**, 1149–1166.
- Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, **180**, 977–993.
- Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J (2008) Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology*, **17**, 167–178.
- Grabowski TB, Young SP, Libungan LA, Steinarrsson A, Marteinsdottir G (2009) Evidence of phenotypic plasticity and local adaption in metabolic rates between components of the Icelandic cod (*Gadus morhua* L.) stock. *Environmental Biology of Fishes*, **86**, 361–370.
- Guillot G, Mortier F, Estoup A (2005) GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes*, **5**, 712–715.
- Hansen MM, Meier K, Mensberg K-LD (2010) Identifying footprints of selection in stocked brown trout populations: a spatio-temporal approach. *Molecular Ecology*, **19**, 1787–1800.
- Hansen MM, Olivieri I, Waller DM, Nielsen EE, The GeM Working Group (2012) Monitoring adaptive genetic responses to environmental change. *Molecular Ecology*, **21**:1311–1329.
- Harrald M, Wright PJ, Neat FC (2010) Substock variation in reproductive traits in North Sea cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 866–876.
- Hedgecock D (1994) Does variance in reproductive success limit effective population sizes of marine organisms? In: *Genetics and Evolution of Aquatic Organisms* (ed Beaumont AR), pp. 122–134. Chapman and Hall, London.
- Hedgecock D, Pudovkin AI (2011) Sweepstakes reproductive success in highly fecund marine fish and shellfish: A review and commentary. *Bulletin of Marine Science*, **87**, 971–1002.
- Helbig J, Mertz G, Pepin P (1992) Environmental influences on the recruitment of Newfoundland/Labrador Cod. *Fisheries Oceanography*, **1**, 39–56.
- Hemmer-Hansen J, Nielsen EE, Meldrup D, Mittelholzer C (2011) Identification of single nucleotide polymorphisms in candidate genes for growth and reproduction in a nonmodel organism; the Atlantic cod, *Gadus morhua*. *Molecular*

- Ecology Resources*, **11**, 71–80.
- Hendry AP, Farrugia TJ, Kinnison MT (2008) Human influences on rates of phenotypic change in wild animal populations. *Molecular Ecology*, **17**, 20–29.
- Hilborn R, Minto-Vera CV (2008) Fisheries-induced changes in growth rates in marine fisheries: are they significant? *Bulletin of Marine Science*, **83**, 95–105.
- Hilborn R, Quinn TP, Schindler DE, Rogers DE (2003) Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 6564–6568.
- Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. *Nature*, **470**, 479–485.
- Hoffmann AA, Willi Y (2008) Detecting genetic responses to environmental change. *Nature Reviews Genetics*, **9**, 421–432.
- Hubert S, Higgins B, Borza T, Bowman S (2010) Development of a SNP resource and a genetic linkage map for Atlantic cod (*Gadus morhua*). *BMC genomics*, **11**, 191.
- Hutchings JA (2005) Life history consequences of overexploitation to population recovery in Northwest Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 824–832.
- Hutchings JA, Swain DP, Rowe S *et al.* (2007) Genetic variation in life-history reaction norms in a marine fish. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **274**, 1693–1699.
- Hutchinson WF (2008) The dangers of ignoring stock complexity in fishery management: the case of the North Sea cod. *Biology Letters*, **4**, 693–695.
- Illumina (2008) GenomeStudio Genotyping Module v1.0 User Guide. *Illumina, Inc.* 184 pp.
- Jakobsdóttir KB, Pardoe H, Magnússon Á *et al.* (2011) Historical changes in genotypic frequencies at the Pantophysin locus in Atlantic cod (*Gadus morhua*) in Icelandic waters: evidence of fisheries-induced selection? *Evolutionary Applications*, **4**, 562–573.
- Jensen LF, Hansen MM, Mensberg KLD, Loeschcke V (2008) Spatially and temporally fluctuating selection at non-MHC immune genes: evidence from TAP polymorphism in populations of brown trout (*Salmo trutta*, L.). *Heredity*, **100**, 79–91.
- Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**, 1403–1405.
- Jørgensen C, Enberg K, Dunlop ES *et al.* (2007) Managing evolving fish stocks. *Science*, **318**, 1247–1248.
- Kingsolver JG, Diamond SE (2011) Phenotypic selection in natural populations: what limits directional selection? *The American naturalist*, **177**, 346–357.
- Kinnison MT, Hendry AP (2001) The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica*, **112–113**, 145–164.
- Knutzen H, Olsen EM, Ciannelli L *et al.* (2007) Egg distribution, bottom topography and small-scale cod population structure in a coastal marine system. *Marine Ecology Progress Series*, **333**, 249–255.
- Kuparinen A, Merilä J (2007) Detecting and managing fisheries-induced evolution. *Trends in Ecology & Evolution*, **22**, 652–659.
- Law R (2007) Fisheries-induced evolution: present status and future directions. *Marine Ecology Progress Series*, **335**, 271–277.
- Leonard JA (2008) Ancient DNA applications for wildlife conservation. *Molecular Ecology*, **17**, 4186–4196.
- Lewontin RC, Krakauer J (1973) Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. *Genetics*, **74**, 175–195.
- Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, **4**, 981–994.
- Marcil J, Swain DP, Hutchings JA (2006) Countergradient variation in body shape between two populations of Atlantic cod (*Gadus morhua*). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **273**, 217–223.
- Marsden CD, Woodroffe R, Mills MGL *et al.* (2012) Spatial and temporal patterns of neutral and adaptive genetic variation in the endangered African wild dog (*Lycaon pictus*). *Molecular Ecology*, **21**, 1379–1393.
- Morrissey MB, Hadfield JD (2012) Directional selection in temporally replicated studies is remarkably consistent. *Evolution*, **66**, 435–442.
- Nielsen EE, Bekkevold D (2012) The memory remains: application of historical DNA for scaling biodiversity loss. *Molecular Ecology*, **21**, 1539–1541.
- Nielsen EE, Hansen MM (2008) Waking the dead: the value of population genetic analyses of historical samples. *Fish and Fisheries*, **9**, 450–461.
- Nielsen EE, Cariani A, Aoidh EM *et al.* (2012) Gene-associated markers provide tools for tackling illegal fishing and false eco-certification. *Nature communications*, **3**, 851.
- Nielsen EE, Hemmer-Hansen J, Poulsen NA *et al.* (2009) Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). *BMC Evolutionary Biology*, **9**, 276.
- Nielsen EE, MacKenzie BR, Magnussen E, Meldrup D (2007) Historical analysis of Pan I in Atlantic cod (*Gadus morhua*): temporal stability of allele frequencies in the southeastern part of the species distribution. *Canadian Journal of Fisheries and Aquatic Sciences*, **64**, 1448–1455.
- Nielsen R (2005) Molecular signatures of natural selection. *Annual Review of Genetics*, **39**, 197–218.
- Olsen EM, Heino M, Lilly GR *et al.* (2004) Maturation trends indicative of rapid evolution preceded the collapse of northern cod. *Nature*, **428**, 932–935.
- Olsen EM, Knutsen H, Gjøsæter J *et al.* (2008) Small-scale biocomplexity in coastal Atlantic cod supporting a Darwinian

- perspective on fisheries management. *Evolutionary Applications*, **1**, 524–533.
- Olsen EM, Lilly GR, Heino M *et al.* (2005) Assessing changes in age and size at maturation in collapsing populations of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 811–823.
- Orsini L, Spanier KI, De Meester L (2012) Genomic signature of natural and anthropogenic stress in wild populations of the waterflea *Daphnia magna*: validation in space, time and experimental evolution. *Molecular Ecology*, **21**, 2160–2175.
- Palumbi SR (2001) Humans as the world's greatest evolutionary force. *Science*, **293**, 1786–1790.
- Pepin P, Helbig J (1997) Distribution and drift of Atlantic cod (*Gadus morhua*) eggs and larvae on the northeast Newfoundland Shelf. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 670–685.
- Poulsen N, Hemmer-Hansen J, Loeschcke V (2011) Microgeographical population structure and adaptation in Atlantic cod *Gadus morhua*. *Marine Ecology Progress Series*, **436**, 231–243.
- Reznick DN, Ghalambor CK (2005) Can commercial fishing cause evolution? Answers from guppies (*Poecilia reticulata*). *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 791–801.
- Robichaud D, Rose GA (2004) Migratory behaviour and range in Atlantic cod: inference from a century of tagging. *Fish and Fisheries*, **5**, 185–214.
- Ruzzante DE, Taggart CT, Cook D (1996) Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort contribution and genetic stability.
- Ruzzante DE, Taggart CT, Doyle RW, Cook D (2001) Stability in the historical pattern of genetic structure of Newfoundland cod (*Gadus morhua*) despite the catastrophic decline in population size from 1964 to 1994. *Conservation Genetics*, **2**, 257–269.
- Ruzzante DE, Taggart CT, Lang S, Cook D (2000) Mixed-stock analysis of Atlantic cod near the Gulf of St. Lawrence based on microsatellite DNA. *Ecological Applications*, **10**, 1090–1109.
- Ryman N (2006) CHIFISH: a computer program testing for genetic heterogeneity at multiple loci using chi-square and Fisher's exact test. *Molecular Ecology Notes*, **6**, 285–287.
- Ryman N, Palm S (2006) POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes*, **6**, 600–602.
- Schindler DE, Hilborn R, Chasco B *et al.* (2010) Population diversity and the portfolio effect in an exploited species. *Nature*, **465**, 609–612.
- Schmidt J (1930) Raceundersøgelser. X Den Atlantiske torske (*Gadus callarias* L.) og dens lokale racer. *Meddelser fra Carlsberg Laboratoriet*, **18**, 1–72.
- Siepielski AM, DiBattista JD, Carlson SM (2009) It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecology Letters*, **12**, 1261–1276.
- Sinclair AF, Swain DP, Hanson JM (2002) Measuring changes in the direction and magnitude of size-selective mortality in a commercial fish population. *Canadian Journal of Fisheries and Aquatic Sciences*, **59**, 361–371.
- Smith TB, Bernatchez L (2008) Evolutionary change in human-altered environments. *Molecular Ecology*, **17**, 1–8.
- Stinchcombe JR, Hoekstra HE (2007) Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. *Heredity*, **100**, 158–170.
- Stockwell CA, Hendry AP, Kinnison MT (2003) Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution*, **18**, 94–101.
- Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 9440–9445.
- Storz JF (2005) Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology*, **14**, 671–688.
- Swain D, Frank K, Maillet G (2001) Delineating stocks of Atlantic cod (*Gadus morhua*) in the Gulf of St Lawrence and Cabot Strait areas using vertebral number. *ICES Journal of Marine Science*, **58**, 253–269.
- Swain DP (2011) Life-history evolution and elevated natural mortality in a population of Atlantic cod (*Gadus morhua*). *Evolutionary Applications*, **4**, 18–29.
- Swain DP, Sinclair AF, Mark Hanson J (2007) Evolutionary response to size-selective mortality in an exploited fish population. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **274**, 1015–1022.
- Therkildsen NO, Nielsen EE, Hüsey K, Meldrup D, Geffen AJ (2010a) Does DNA extraction affect the physical and chemical composition of historical cod (*Gadus morhua*) otoliths? *ICES Journal of Marine Science*, **67**, 1251–1259.
- Therkildsen NO, Nielsen EE, Swain DP, Pedersen JS (2010b) Large effective population size and temporal genetic stability in Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 1585–1595.
- Trippel EA (1995) Age at maturity as a stress indicator in fisheries. *Bioscience*, **45**, 759–771.
- Umina PA, Weeks AR, Kearney MR, McKechnie SW, Hoffmann AA (2005) A rapid shift in a classic clinal pattern in *Drosophila* reflecting climate change. *Science*, **308**, 691–693.
- Wandeler P, Hoeck PEA, Keller LF (2007) Back to the future: museum specimens in population genetics. *Trends in Ecology & Evolution*, **22**, 634–642.
- Wang JL (2001) A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genetical Research*, **78**, 243–257.
- Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, **15**, 1419–1439.
- Warnes G (2003) The genetics package. *R News*, **3**, 9–13.

- Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, **163**, 1177–1191.
- Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.

FIGURE LEGENDS

Fig 1. Map showing the approximate sampling locations for the four populations (blue dots). Dashed lines delimit Northwest Atlantic Fishery Organization (NAFO) management areas for cod in Canada and grey solid lines are the 200 and 1000 m isobaths.

Fig. 2. Results from the temporal outlier tests in 4T 1928-2008 (2 samples; a), 4T 1960-2002 (5 samples; b), 3NO 1960-2010 (4 samples; c), 3Ps 1964-2010 (3 samples; d), and 4VsW 1964-2010 (2 samples; e). Each dot represents a locus, illustrating its temporal differentiation (F_{temp} , y-axis) against its mean heterozygosity (H_s , x-axis). The lines represent the 95% (grey) and the 99% (black) confidence envelopes of the simulated neutral distribution. Significant outliers after FDR correction ($q < 0.05$) are circled in red.

Fig. 3. Observed allele frequencies at the significant 4T 1928-2008 outlier loci in different sampling years in 4T (a), 3NO (b), 3Ps (c), and 4VsW (d). Outlier loci are plotted in different shades of green and each dot represents a sample. Dots are connected with lines for easier visualization of temporal trends.

Fig. 4. Results from the spatial outlier tests in the 1960s (a), the 1980s-1990s (b), and the 2000s (c). Tests for the 1960s and 2000s are based on all four populations while the 1980s-1990s test is based on 4T, 3NO and 3Ps only. Each dot represents a locus, illustrating its spatial differentiation (F_{ST} , y-axis) against its mean heterozygosity (H_e , x-axis). The lines represent the 95% (grey) and the 99% (black) confidence envelopes of the simulated neutral distribution. Significant outliers after FDR correction ($q < 0.05$) are circled in red.

Fig. 5. Observed allele frequencies at temporal outlier loci in different sampling years within 4T plotted with temporal trends in ambient fall temperature for cod (a), and probabilistic maturation reaction norm midpoint (b). Loci in linkage group 12 are plotted in different shades of blue and loci in linkage group 1 in different shades of red and orange. Dots represent samples and they are connected with lines for easier visualization of temporal trends.

AUTHOR CONTRIBUTIONS BOX

NOT and EEN designed the research with input from DPS, MJM, and EAT. DPS, MJM, and EAT contributed samples. NOT and DM performed the laboratory research. NOT analyzed the data with input from EEN, SRP, TDA, and JHH. NOT and EEN wrote the paper with input from all other authors.

Table 1. Sampling years for each population and sample size (n), number of loci genotyped (# loci), the proportion of loci that were polymorphic (% variable), and the observed (H_{obs}) and expected (H_e) heterozygosity for each sample

Population	Year	n*	# loci	% variable	H_{obs}	H_e
4T	1928	29	1047	0.87	0.27	0.28
	1960	37	160	0.84	0.25	0.26
	1968	31	160	0.84	0.28	0.27
	1974**	14	160	0.78	0.27	0.26
	1976	36	160	0.85	0.28	0.27
	1983	37	160	0.86	0.28	0.28
	2002	29	160	0.84	0.28	0.26
	2008	39	1047	0.86	0.28	0.28
3NO	1960	28	160	0.82	0.25	0.26
	1973	37	160	0.85	0.26	0.26
	1990	37	160	0.86	0.25	0.25
	2010	32	160	0.87	0.27	0.26
3Ps	1964	33	160	0.82	0.25	0.25
	1993	25	160	0.84	0.26	0.25
	2010	26	160	0.86	0.28	0.27
4VsW	1961	16	160	0.86	0.27	0.26
	2010	22	160	0.92	0.28	0.28

*Sample sizes represent the number of individuals included in the analysis (i.e. excluding samples that did not pass the quality filtering criteria)

** Due to the small n, this sample was pooled with the 1976 sample for analysis (there was no significant difference in allele frequencies between 1974 and 1976)

Table 2. Summary of significant results in temporal and spatial outlier tests on the 1960-2010 samples. 95 and 99 indicate that the locus was above the 95% or the 99% confidence limits, respectively, in ftemp or fdist analysis. * indicates that the outlier remained significant following FDR control ($q < 0.05$), BS3 and BS10 indicate that the locus was an outlier in BayeScan analysis with prior odds favoring the neutral model of 3 and 10, respectively.

SNP Name	LG	Temporal outliers				Spatial outliers		
		3NO	3Ps	4T	4VsW	1960s	1980-1990*	2000s
Rhod_1_1	1	99*, BS3*	95		99	99*	95, BS3	
cgpGmo-S1874	1	99*	95		95	99	95, BS3	
cgpGmo-S1955	1	99*	95		95	99	95, BS3	
cgpGmo-S1166	1	99*	95			99	99, BS3	
cgpGmo-S985	1	99*	95			99	95	
Pan1	1	99*	99			95	95	
cgpGmo-S1456	2	99*				95		
cgpGmo-S1101a	2				95			99
cgpGmo-S1068	2							99
cgpGmo-S1970	5				95			
cgpGmo-S1200	7	99*		95			99*	
cgpGmo-S1017	9	95						
LDHB	9	95						
cgpGmo-S1737	12		99*, BS3*		99*		99	99*
cgpGmo-S180b	12		99*, BS10*		99		99*, BS10*	99*, BS10*
cgpGmo-S816a	12		99*, BS10*		95		99*, BS10	99*, BS3
cgpGmo-S866	12		99*, BS10		95		99, BS3	99, BS3*
cgpGmo-S57	12		99*, BS10		95		95	
cgpGmo-S2101	12		99*					
cgpGmo-S1046	12		99*, BS3				95	95
cgpGmo-S316	12				95		95	
cgpGmo-S142	14	95						
cgpGmo-S1467	14			95				
cgpGmo-S955	17	99*						
cgpGmo-S1340	18				99			
cgpGmo-S442a	18				95			
Gm370_0380	22	95						
Anti_1	22		95		95			
Gm0588_0274	?	99*						
cgpGmo-S1406	?			95			99*	
cgpGmo-S1731	?				95			
Gm335_0159	?							99*
Total number of outliers		14	7	10	13	7	15	8
Total with FDR control		10	0	7	1	1	4	5

*This comparison does not include 4VsW

Table 3. Results from the AMOVA based on all loci (n=157) and non-outlier loci only (n=101)

Data	Source of variation	F-index	df	Var components	% variation (95% CI)	p-value
All loci	Among populations	F_{CT}	3	0.10	0.44 (0.21 - 0.68)	<0.00001
	Among time points within populations	F_{SC}	12	0.12	0.54 (0.28 - 0.83)	<0.00001
	Among individuals within time points	F_{IS}	463	0.22	1.03 (-0.41 - 2.67)	0.01059
	Within individuals			21.35	97.99	
Non-outlier loci	Among populations	F_{CT}	3	0.02	0.15 (0.024 - 0.27)	0.00639
	Among time points within populations	F_{SC}	12	-0.01	-0.09 (-0.24 - 0.064)	0.86560
	Among individuals within time points	F_{IS}	463	0.19	1.14 (-0.58 - 3.156)	0.01269
	Within individuals			16.16	98.80	

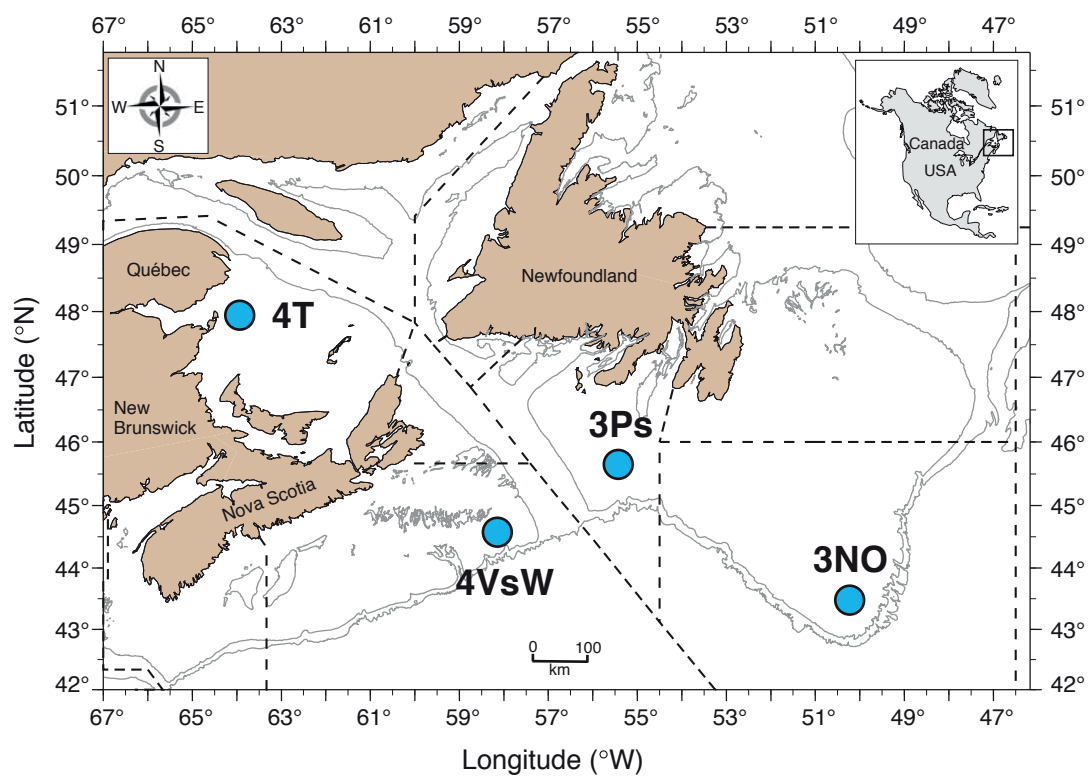


Fig. 1

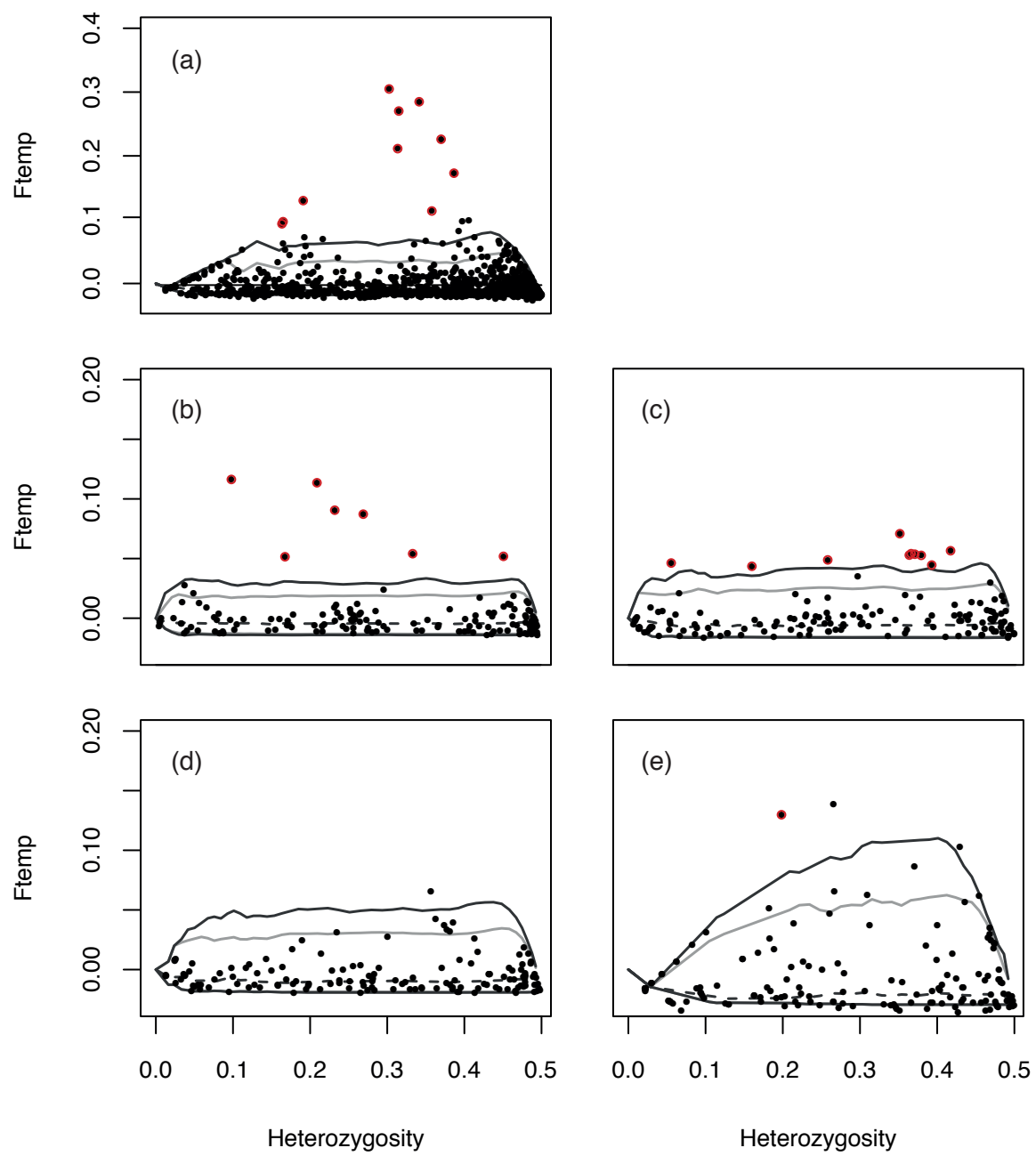


Fig. 2

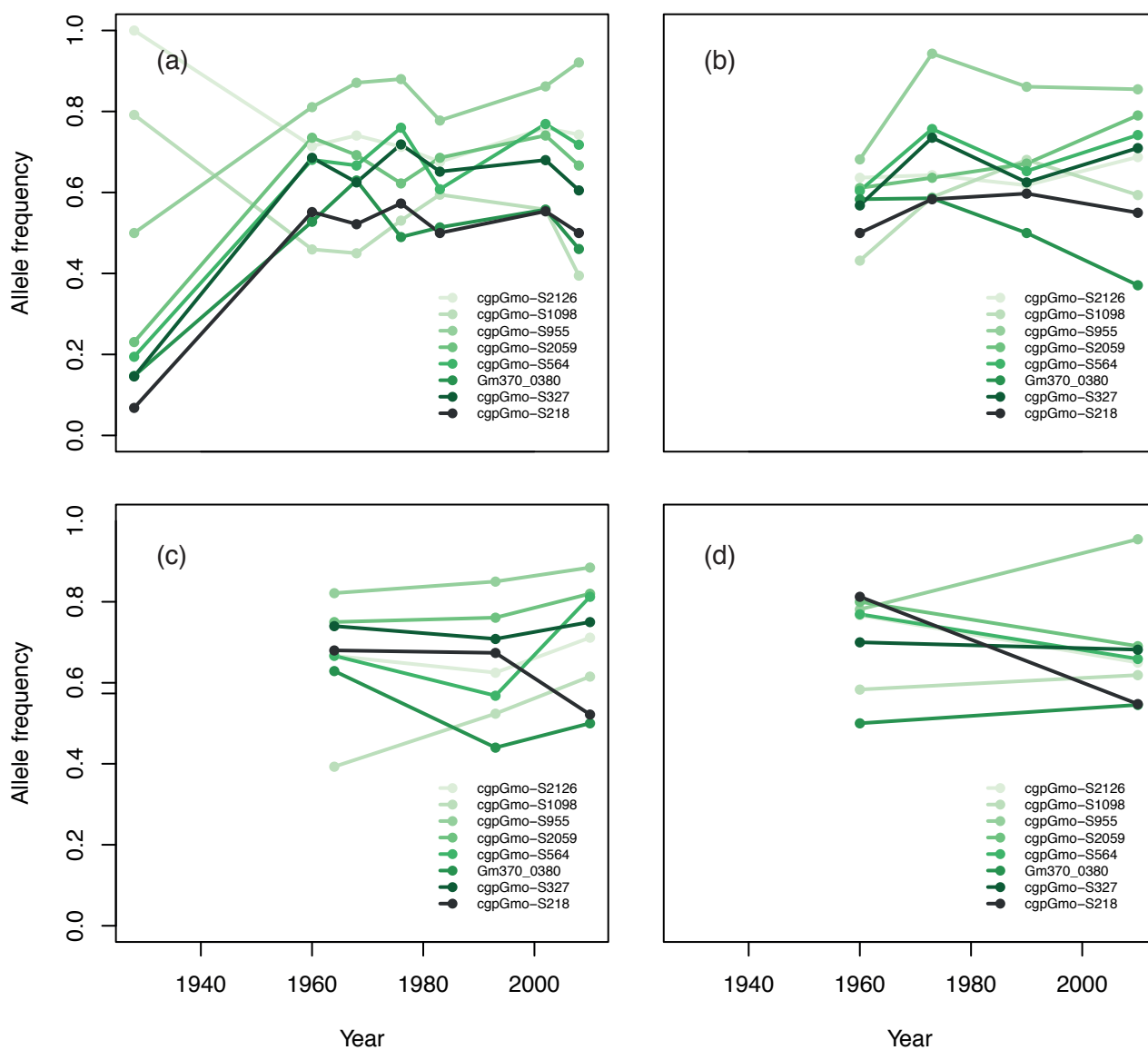


Fig. 3

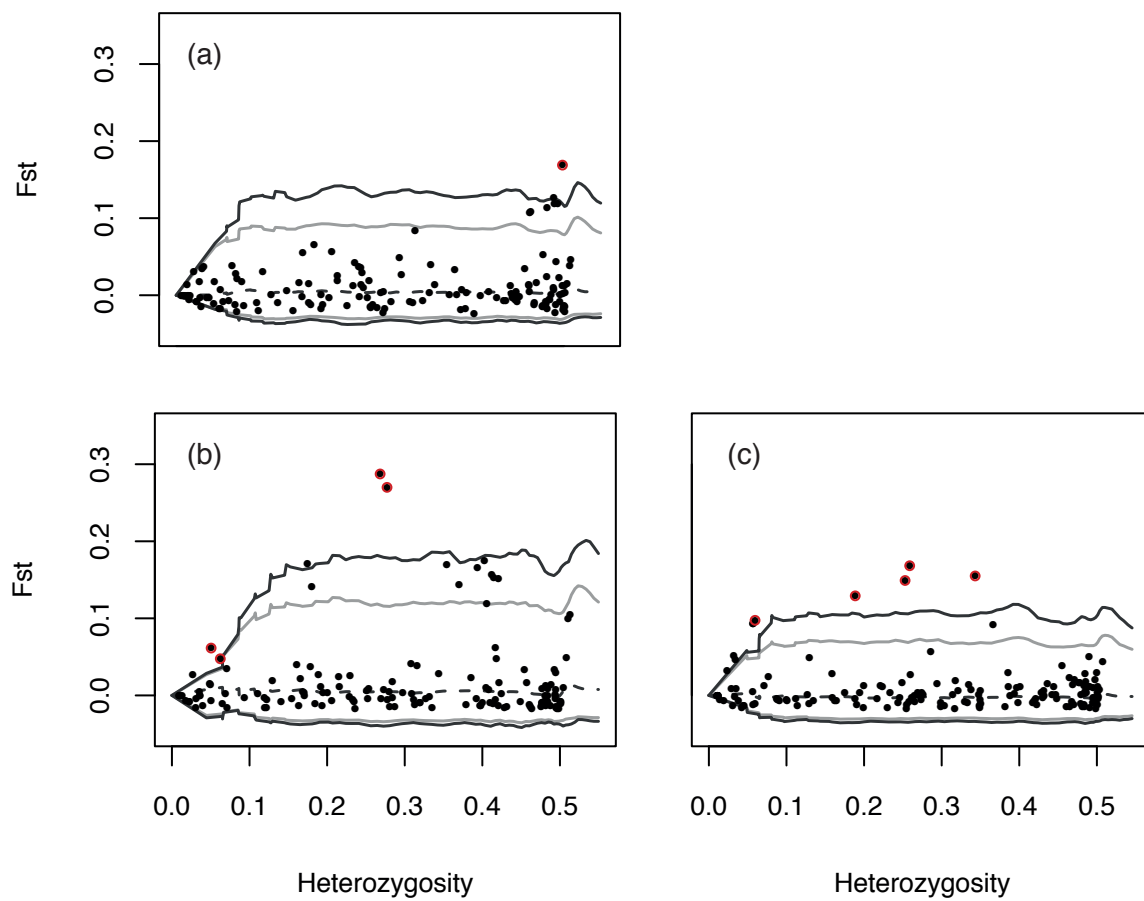


Fig. 4

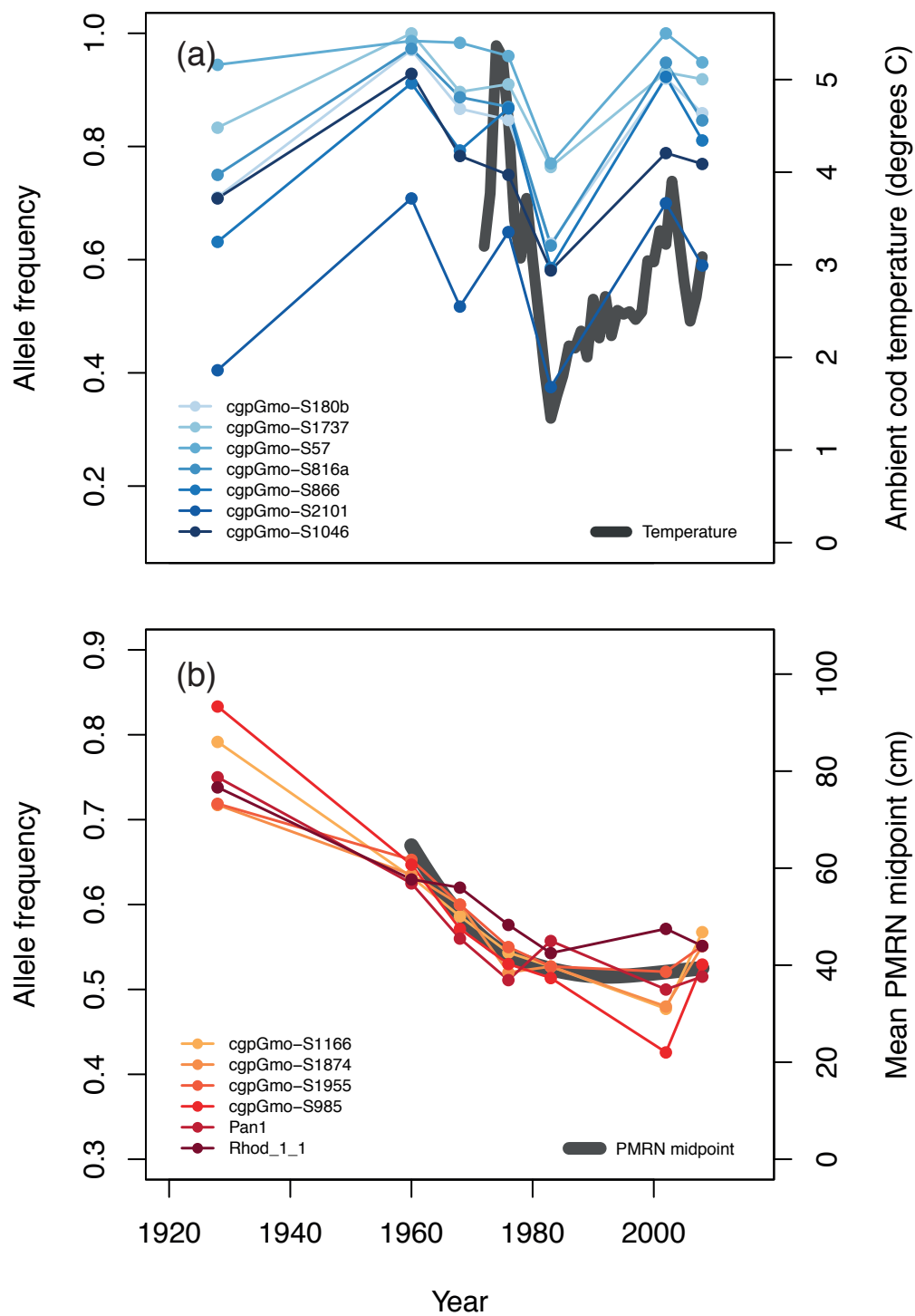


Fig. 5

Supporting information for the article

Microevolution in time and space: SNP analysis of historical DNA reveals dynamic signatures of selection in Atlantic cod

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This file contains:

Supplementary Methods/Results

Note 1: Genomic location and annotation of SNPs

Note 2: Temporal outlier detection

Note 3: Simulation of migration between populations

References

Supplementary Tables

Tables S1-S6

Supplementary Figures

Figures S1-S10

Supplementary Methods/Results

Note 1: Genomic location and annotation of SNPs

Approach

In total 985 of the 1536 study SNPs were already positioned on the cod linkage map (Hubert *et al.* 2010; Borza *et al.* 2010). By mapping 120 bp of the flanking sequence surrounding each SNP on to scaffolds of the cod genome sequence (Star *et al.* 2011) using BLASTN with an e-value threshold of 10^{-10} , we could anchor an additional set of 489 SNPs (for a total of 1474) to the linkage map. The SNPs were fairly evenly distributed among the 23 linkage groups (with between 34 and 97 (mean of 64) SNPs per linkage group), ensuring broad genomic coverage in our initial scan.

The expected functional role was already described for the candidate gene SNPs (Hemmer-Hansen *et al.* 2011), but for the remainder, we queried the 120 bp flanking sequence for each SNP against NCBI's NR database and Uniprot's SwissProt and TrEMBL databases using BLASTX with an e-value threshold of 10^{-5} . Only ~30% of sequences showed significant homology to known proteins, probably because they were primarily developed from the 3' end of ESTs and many are therefore located in non-coding untranslated regions (Hubert *et al.* 2010). However, even if the SNPs are not direct targets of selection, they may be affected by hitchhiking effects from selection acting on closely linked sites (Smith & Haigh 2007). Therefore, we examined annotation information for the surrounding genomic region of each SNP by mapping (with BLASTN using an e-value threshold of 10^{-10}) the flanking sequence onto the Ensembl Atlantic cod genome assembly (www.ensembl.org; release 65, Dec 2011). This assembly has been annotated using a combination of the standard Ensembl pipeline and a complimentary method based on aligning re-organized scaffolds to the stickleback (*Gasterosteus aculeatus*) genome (Star *et al.* 2011). We extracted data, including gene ontology (GO) terms, for all gene models located within 10 kb up- or downstream of each SNP. To summarize the functional information associated with outlier SNPs, the online tool CateGORizer (Hu *et al.* 2008) was used to cluster the extracted GO terms according to the MGI GOSlim2 classification method (single occurrence counting). For the SNPs that were located within coding regions of the gene models, we determined whether the substitution caused an amino acid change or not following the approach used by Milano *et al.* (2011).

Results for outlier SNPs

All post-1928 outlier loci showed significant matches to the annotated genome assembly (all but two mapped uniquely to single GeneScaffolds). Only 11 of these outlier SNPs were located directly within gene models and 6 of these within exons (all synonymous substitutions). However, the +/- 10 kb flanking region contained between 1 and 5 gene models for all outlier SNPs, adding up to a total of 80 gene models represented by the outlier loci (Table S3). These genes were associated with a total of 239 GO terms (146 unique terms), representing a broad variety of functional roles. Classification of the GO terms into MGI GOSlim2 overview categories revealed that the most frequently represented summary categories among the outlier loci were metabolism and developmental processes (Table S4).

ST2: Temporal outlier detection

Approach

To identify loci that showed elevated levels of differentiation among samples collected over time within single populations, we used a modified version of the *fdist* method (Beaumont & Nichols 1996) that is commonly applied for this purpose in spatial comparisons of samples collected from different populations at a single time point. Based on the premise that selection should affect only certain parts of the genome whereas neutral evolutionary forces should cause genome-wide effects, the method compares the observed locus-specific F_{ST} values as a function of heterozygosity (H_s) to a null distribution generated through simulations. Any loci that show divergent patterns of differentiation compared to this neutral expectation are then considered a candidate for being affected by selection.

Here, we adapted the method to fit our scenario by generating the expected neutral distribution through simulation of drift within a single population rather than as drift-migration equilibrium between multiple demes, as is implemented in the original formulation. Migration can have contrasting effects on allele frequencies within a population over short time scales depending on the level of differentiation between the source and the recipient populations (Wang & Whitlock 2003; Fraser *et al.* 2007) and this can be complex to generalize. Consequently, our null model included only the effects of drift and sampling within an isolated population. Assuming that the time scale considered in this study (up to 12 generations) is sufficiently short to ignore the effects of mutations, any departure from the null model expectations is then likely caused either by selection or gene flow.

Model and parameter inputs

Our simulations were based on single bi-allelic loci at initial frequency f_0 in a Wright-Fisher population of constant size, N_e , that reproduced over t_{max} generations. At each t_s generation, a sample of size n individuals were collected. We ran the analysis separately for each of the four populations, each time parameterizing the model to most closely match the studied scenario.

The initial allele frequency f_0 at each simulated locus was a random number between 0 and 1, but to generate a roughly uniform distribution of H_s values among the simulated loci, we enriched for low starting frequencies. We used the pseudo-likelihood estimator of Wang (2001) as implemented in the software MLNE (Wang & Whitlock 2003) to estimate the N_e for each population based on temporal shifts in allele frequencies. To match the simulation assumptions, we used the closed population model (i.e. no migration) and set N_{eMAX} to 30,000, the maximum value the program could handle on a standard computer. Because the N_e is a difficult parameter to estimate, particularly for large populations (Hare *et al.* 2011; Palstra & Ruzzante 2011), we took a conservative approach and used the lower 95% confidence interval limit on estimates as input for the simulations. Further, to minimize downward bias from inclusion of loci potentially under selection (Wang 2005; Leberg 2005), we ran the analysis iteratively: First we identified outlier loci from preliminary simulations with an N_e estimate based on all loci. We then re-estimated the N_e based only on non-outlier loci and used this second estimate as input for the final simulations.

We estimated the generation time for each of the four populations as the mean age of spawners based on survey data on abundance, age distribution, and fecundity at age following the approach in Therkildsen *et al.* (2010). We then considered our first sample from each population generation 0 and converted years between subsequent sampling points to generations (rounded to integers).

T_{max} was the generation where the contemporary sample was collected and each preceding sample represented a generation t_s . The sample size n was the harmonic mean of sample sizes for the population. Using these parameter inputs, we obtained time series of observed allele frequencies for the simulated loci that corresponded to the sampling pattern in the real data.

Outlier identification

We quantified the temporal variance in allele frequencies F_{temp} between all samples from a population in both the observed and simulated data with Wright's F (Wright 1951), correcting for sampling effects following Waples (1998):

$$F_{temp} = \frac{Var(p)}{\bar{p}(1 - \bar{p})} - \frac{1}{2n}$$

The correction for sampling effects was important because missing data made the actual sample size vary between loci in the observed data. Following Beaumont and Nichols (1996), we plotted F_{temp} as a function of the H_s for each locus. We simulated 100,000 independent loci and for each computed paired values of F_{temp} and H_s . As in the *fdist* method, the paired values were rank-ordered by H_s and grouped into overlapping bins of 4,000 points centered on every 2,000th point. For each bin, we computed the quantiles of the distribution of F_{temp} values that would define the confidence envelopes in which 95% and 99%, respectively, of the data points were expected to lie if behaving according to the model. To assess the statistical significance of departures from the neutral expectation, empirical p-values were computed for each locus as the proportion of simulated data points within its bin that showed higher F_{temp} than the observed value. To control the false discovery rate to <5%, we also computed q-values for all loci using the R-package *qvalue* (Storey & Tibshirani 2003). All simulations and computations were completed with custom R-scripts (available upon request).

Since both the N_e and the generation length are difficult to estimate in wild populations, we evaluated the sensitivity of the method to uncertainty in these parameter inputs. Basing simulations on point estimates instead of the lower confidence interval limits for N_e generally caused no difference to which loci were identified as outliers (only one locus more or less in a few cases). Similarly, almost identical results were obtained when varying the generation time estimate (Table S2) by +/- 2 years. This stability indicates that our conclusions are robust to uncertainty in input parameters.

ST3: Simulation of migration between populations

To evaluate whether migration between the populations could have caused the observed temporal fluctuations at outlier loci, we conducted a series of simple simulations involving various levels of exchange. For one population at a time (the focal population), we used a Wright-Fisher model of size N_e similar to the model adopted for the temporal outlier detection above, but here we for each generation let $N_e m$ individuals originate from a separate source population rather than the focal population. In each simulated data set, we recorded allele frequencies at 160 loci corresponding to the real data. Treating the 1960s samples as baselines, we used the observed allele frequencies in these samples (assuming no sampling error) as initial frequencies for our focal and source populations. For each focal population, we conducted separate simulations with each of the three other populations as the migrant source, each time sampling the simulated focal population at the generations and with the sample sizes that matched the observed data.

We then compared these simulated data to the null distribution of expected differentiation in the absence of selection and gene flow (generated for the focal population in Supporting information Note 2) to identify the total number of temporal outliers (regardless of locus identity) as well as the proportion of the actual temporal outlier loci from the observed data that also became temporal outliers in the simulations. Because of uncertainty in N_e estimates, we ran the simulations for focal population N_e ranging from 100 to 100,000 and varied m from 0 to 1 in steps of 0.1, using 100 simulated datasets for each combination of values. The simulations were conducted with custom R-scripts available upon request.

References

- Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **263**, 1619–1626.
- Borza T, Higgins B, Simpson G, Bowman S (2010) Integrating the markers Pan I and haemoglobin with the genetic linkage map of Atlantic cod (*Gadus morhua*). *BMC Research Notes*, **3**, 261.
- Fraser DJ, Hansen MM, Østergaard S *et al.* (2007) Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. *Molecular Ecology*, **16**, 3866–3889.
- Hare MP, Nuney L, Schwartz MK *et al.* (2011) Understanding and estimating effective population size for practical application in marine species management. *Conservation Biology*, **25**, 438–449.
- Hemmer-Hansen J, Nielsen EE, Meldrup D, Mittelholzer C (2011) Identification of single nucleotide polymorphisms in candidate genes for growth and reproduction in a nonmodel organism; the Atlantic cod, *Gadus morhua*. *Molecular Ecology Resources*, **11**, 71–80.
- Hu Z-L, Bao J, Reecy J (2008) CateGORizer: A web-based program to batch analyze gene ontology classification categories. *Online Journal of Bioinformatics*, **9**, 108–112.
- Hubert JP, Higgins B, Borza T, Bowman S (2010) Development of a SNP resource and a genetic linkage map for Atlantic cod (*Gadus morhua*). *BMC genomics*, **11**, 191.
- Leberg P (2005) Genetic approaches for estimating the effective size of populations. *Journal of Wildlife Management*, **69**, 1385–1399.
- Milano I, Babbucci M, Panitz F *et al.* (2011) Novel tools for conservation genomics: Comparing two high-throughput approaches for SNP discovery in the transcriptome of the European hake. *PLoS ONE*, **6**, e28008.
- Palstra FP, Ruzzante DE (2011) Demographic and genetic factors shaping contemporary metapopulation effective size and its empirical estimation in salmonid fish. *Heredity*, **107**, 444–455.
- Smith JM, Haigh J (2007) The hitch-hiking effect of a favourable gene. *Genetical Research*, **89**, 391–403.
- Star B, Nederbragt AJ, Jentoft S *et al.* (2011) The genome sequence of Atlantic cod reveals a unique immune system. *Nature*, **477**, 207–210.
- Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 9440–9445.
- Therkildsen NO, Nielsen EE, Swain DP, Pedersen JS (2010) Large effective population size and temporal genetic stability in Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 1585–1595.
- Wang J (2005) Estimation of effective population sizes from data on genetic markers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **360**, 1395–1409.
- Wang JL (2001) A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genetical Research*, **78**, 243–257.
- Wang JL, Whitlock MC (2003) Estimating effective population size and migration rates from genetic samples over space and time. *Genetics*, **163**, 429–446.
- Waples RS (1998) Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, **89**, 438–450.
- Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–353.

Table S1. List of environmental variables used in the correlation analysis with temporal outlier loci in 4T

Variable abbreviation	Variable description	Earliest year	Data source	Note
PMRN midpoint	The mean probabilistic maturation reaction norm midpoint (mean across sexes and ages 4-5)	1960	Swain 2011	This measure filters away growth-related phenotypic plasticity from maturation trends
Weight-at-age	The average weight-at-age for fish age 5-7 as a proxy for adult growth rate	1960	Table 13, Swain et al. 2009	The mean of the annual estimates for the age classes 5-7 was selected because these are the ages best represented in the samples
Biomass	The total biomass of fish age 3+ as a proxy for population density	1950	DFO 2011	There is little fishing on younger age classes
M age 2-4	The natural mortality rate for age classes 2-4 (juveniles)	1950	DFO 2011	There is little fishing on younger age classes
M age 5+	The natural mortality rate for age classes ≥ 5 (adults)	1950	DFO 2011	This water mass covers the bottom throughout most of the southern Gulf of St Lawrence and is considered a general index of oceanographic conditions in the area
F age 5+	The fishing mortality rate for age classes ≥ 5 (adults)	1950	DFO 2011	This water mass covers the bottom throughout most of the southern Gulf of St Lawrence and is considered a general index of oceanographic conditions in the area
Z age 5+	The total mortality rate (natural and fishing) for age classes ≥ 5 (adults)	1950	DFO 2011	This water mass covers the bottom throughout most of the southern Gulf of St Lawrence and is considered a general index of oceanographic conditions in the area
CIL temp mean	An index of the mean recorded temperature in the 60-120 m water mass (cold intermediate layer) in September	1960	Galbraith et al. 2011	This water mass covers the bottom throughout most of the southern Gulf of St Lawrence and is considered a general index of oceanographic conditions in the area
CIL temp min	An index of the minimum recorded temperature in the 60-120 m water mass (cold intermediate layer) in September	1960	Galbraith et al. 2011	This water mass covers the bottom throughout most of the southern Gulf of St Lawrence and is considered a general index of oceanographic conditions in the area
CIL temp max	An index of the maximum recorded temperature in the 60-120 m water mass (cold intermediate layer) in September	1960	Galbraith et al. 2011	This water mass covers the bottom throughout most of the southern Gulf of St Lawrence and is considered a general index of oceanographic conditions in the area
Cod temp mean	An index of the ambient temperature experienced by cod in September derived by weighting spatially explicit temperature measurements by local cod abundance	1971	Swain et al. 2007	The measure has been updated from the original publication to include 2008. It is calculated by aggregating over all ages

References:

- DFO. 2011. Recovery Potential Assessment for the Laurentian South Designatable Unit of Atlantic Cod (*Gadus morhua*). DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2011/028.
- Galbraith, P.S., Chassé, J., Gilbert, D., Larouche, P., Brickman, D., Pettigrew, B., Devine, L., Gosselin, A., Pettipas, R.G. and Lafleur, C., 2011. Physical Oceanographic Conditions in the Gulf of St. Lawrence in 2010. DFO Can. Sci. Advis. Sec. Res. Doc. 2011/045. iv + 82 p.
- Swain DP, Sinclair AF, Mark Hanson J (2007) Evolutionary response to size-selective mortality in an exploited fish population. Proceedings of the Royal Society of London Series B-Biological Sciences, 274, 1015–1022.
- Swain, D.P., Savole, L., Hurlbut, T., Surette, T., Daigle, D. 2009. Assessment of the southern Gulf of St. Lawrence cod stock, February 2009. DFO Can. Sci. Advis. Sec. Res. Doc. 2009/037. vi + 129 p.
- Swain DP (2010) Life-history evolution and elevated natural mortality in a population of Atlantic cod (*Gadus morhua*). Evolutionary Applications, 4, 18–29.

Table S2. Estimates of generation length, generations sampled in the study, and the effective population size (N_e) and its 95% confidence interval (CI) for each population. All N_e estimates are based on a subset of loci that exclude temporal outliers for the given population. For 4T separate estimates are presented for the 1928-2008 and the 1960-2002 samples due the different number of loci. Inf indicates N_e estimates of infinity

Population	Generation length	Generations sampled	N_e (95% CI)
3NO	8.1	0, 2, 4, 7	Inf (509-Inf)
3Ps	7.8	0, 4, 6	Inf (619-Inf)
4VsW	6.4*	0, 8	Inf (452-Inf)
4T 1960-2002	6.4	0, 1, 2, 3, 6	Inf (568-Inf)
4T 1928-2008	6.4	0, 12	1576 (820-9045)

* Assumed to be identical to 4T because 4VsW and 4T have similar generation length estimates in Table A1 of the Committee on the Status of Endangered Wildlife in Canada (2010) COSEWIC assessment and update status report on the Atlantic cod (*Gadus morhua*) in Canada

Table S3. List of gene models located within 10kb up- or downstream of each outlier SNP. "LG" is the linkage group and "Position" indicates the position within the linkage group for each SNP. "Gene name" is the name of gene models extracted from the Ensembl annotated genome assembly. "Dist to gene" indicates the number of bases from the SNP position to the beginning of the gene model. Where SNPs are located within gene models, "Substitution" indicates whether the SNP is located within an intron or causes a synonymous (S) or non-synonymous (N) amino acid change. "Gene description" provides the Ensembl gene model descriptor. "Status gene" indicates whether the gene model refers to a known, novel or known-by-projection (kbp) gene. "GOSlim description" provides terms associated with the gene model.

SNP Name	LG	Position	Gene Name	Dist to gene	Substitution	Gene description	Status gene	GOSlim description
cgpGmo-S985	1	15.6	ENSGMORG000000003958	512		solute carrier family 25, member 33 [Source:HGNC Symbol;Acc:29681]	kbp	
			ENSGMORG000000003979	2572			novel	cell, cellular_component
			ENSGMORG000000003991	7905			novel	nuclear envelope, organelle, nucleus, cell, intracellular, cellular_component
Pan1	1	25.1	ENSGMORG000000001141	1430		ataxin 7-like 2 [Source:HGNC Symbol;Acc:28713]	kbp	
			ENSGMORG000000001154	0	intron	Pantophysin [Source:UniProtKB/TrEMBL;Acc:Q98SD9]	known	transport, cytoplasmic membrane-bounded vesicle, organelle, cytoplasm, cell, intracellular, cellular_component
			ENSGMORG000000001169	3770		sortilin 1 [Source:HGNC Symbol;Acc:11186]	kbp	
cgpGmo-S1166	1		ENSGMORG000000008246	4816		La ribonucleoprotein domain family, member 4 [Source:HGNC Symbol;Acc:24320]	kbp	
			ENSGMORG000000008251	573		ankyrin repeat and SOCS box containing 8 [Source:HGNC Symbol;Acc:17183]	kbp	signal transduction
			ENSGMORG000000008255	9794		activating transcription factor 7 [Source:HGNC Symbol;Acc:792]	kbp	cell, intracellular, cellular_component
cgpGmo-S1874	1		ENSGMORG000000001067	704		guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 2 [Source:HGNC Symbol;Acc:4394]	kbp	signal transduction
Rhod_1_1	1		ENSGMORG000000001092	3786		membrane associated guanylate kinase, WW and PDZ domain containing 1 [Source:HGNC Symbol;Acc:946]	novel	signal transduction, cell, intracellular, cellular_component
			ENSGMORG0000000016734	4970			kbp	
			ENSGMORG0000000016805	155			novel	neurological system process, protein modification process, signal transduction, cell, cellular_component
			ENSGMORG0000000020380	0	S	Rhodopsin [Source:UniProtKB/TrEMBL;Acc:Q5K6H7]	known	cell, cellular_component
cgpGmo-S1456	2	49.7	ENSGMORG0000000014698	0	unknown	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2 [Source:HGNC Symbol;Acc:6305]	novel	
			ENSGMORG0000000014715	87		plexin domain containing 1 [Source:HGNC Symbol;Acc:20945]	kbp	cell adhesion, cell, cellular_component
			ENSGMORG0000000014729	8023		Symbol;Acc:20945]	kbp	
			ENSGMORG0000000017164	1036		DBB1 and CUL4 associated factor 7 [Source:HGNC Symbol;Acc:30915]	kbp	
			ENSGMORG0000000009936	2408		sodium channel, voltage-gated, type IV, alpha subunit [Source:HGNC Symbol;Acc:10591]	kbp	transmembrane transport, transport, plasma membrane, protein complex, cell, cellular_component
cgpGmo-S1970	5		ENSGMORG0000000012789	8072		tumor protein p53 inducible protein 3 [Source:HGNC Symbol;Acc:19373]	kbp	
			ENSGMORG0000000012794	6374		DnaJ (Hsp40) homolog, subfamily C, member 5 gamma [Source:HGNC Symbol;Acc:24844]	kbp	
			ENSGMORG0000000012796	1371			novel	transmembrane transport, transport, cell, cellular_component
			ENSGMORG0000000012801	8579		TatD DNase domain containing 3 [Source:HGNC Symbol;Acc:27010]	kbp	
cgpGmo-S1200	7	2.9	ENSGMORG0000000012350	4644		DEAH (Asp-Glu-Ala-His) box polypeptide 33 [Source:HGNC Symbol;Acc:16718]	kbp	
			ENSGMORG0000000012353	196		complement component 1, q subcomponent binding protein [Source:HGNC Symbol;Acc:1243]	kbp	mitochondrion, organelle, cytoplasm, cell, intracellular, cellular_component

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Table S3 -continued

[illegible]

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Table S3 -continued

cgpGmo-S2101	12	32.2	ENSGMOG000000005901	2830		protein phosphatase 1, regulatory (inhibitor) subunit 2 [Source:HGNC Symbol;Acc:9288] ArfGAP with coiled-coil, ankyrin repeat and PH domains 2 [Source:HGNC Symbol;Acc:16469] mitogen-activated protein kinase 14 [Source:HGNC Symbol;Acc:6876] calmodulin-like 4 [Source:HGNC Symbol;Acc:18445] ceroid-lipofuscinosis, neuronal 6, late infantile, variant [Source:HGNC Symbol;Acc:2077] transmembrane protein 55B [Source:HGNC Symbol;Acc:19299] ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1) [Source:HGNC Symbol;Acc:9801]	kbp kbp kbp kbp kbp novel kbp	protein modification process cell, intracellular, cellular_component
cgpGmo-S241	13	43.1	ENSGMOG000000004454	2607			kbp	
cgpGmo-S1467	14	17.9	ENSGMOG000000008451 ENSGMOG000000008452	325 480			kbp kbp	
cgpGmo-S142 cgpGmo-S955	14 17	45.4 34.1	ENSGMOG000000001070 ENSGMOG000000002376	4359 944			novel kbp	
cgpGmo-S442a	18	27.7	ENSGMOG000000002652	855			kbp	nucleocytoplasmic transport, nucleobase, nucleoside, nucleotide and nucleic acid catabolic process, transport, signal transduction, small molecule metabolic process, catabolic process, cell, intracellular, cellular_component, cellular nitrogen compound metabolic process
cgpGmo-S1340	18	32.7	ENSGMOG000000002670	988		crystallin, mu [Source:HGNC Symbol;Acc:2418]	kbp	
			ENSGMOG000000002685	5506		NHP2 non-histone chromosome protein 2-like 1 (S. cerevisiae) [Source:HGNC Symbol;Acc:7819] ubiquinol-cytochrome c reductase core protein II [Source:HGNC Symbol;Acc:12586]	kbp kbp	ribosome biogenesis, cell, intracellular, cellular_component
			ENSGMOG000000002698	6974			kbp	
			ENSGMOG0000000011582 ENSGMOG0000000011594	1230 162		COP9 constitutive photomorphogenic homolog subunit 3 (Arabidopsis) [Source:HGNC Symbol;Acc:2239] 5', 3'-nucleotidase, cytosolic [Source:HGNC Symbol;Acc:17144]	novel kbp	catabolic process
			ENSGMOG0000000011603	7619			kbp	
I"#%&%%%	22		ENSGMOG0000000011985 ENSGMOG0000000011990	5026 0	S	ribosomal protein L11 [Source:HGNC Symbol;Acc:10301]	novel kbp	
			ENSGMOG0000000011995	6363			novel	ribosome, translation, organelle, biosynthetic process, cytoplasm, cell, intracellular, cellular_component translation, biosynthetic process, cytoplasm, cell, intracellular, cellular_component
Anti_1	22		ENSGMOG000000002448 ENSGMOG000000002484 ENSGMOG000000002496	2293 0 4457	S	Lix1 homolog (mouse)-like [Source:HGNC Symbol;Acc:28715] phospholipase C, gamma 1 [Source:HGNC Symbol;Acc:9065] topoisomerase (DNA) I [Source:HGNC Symbol;Acc:11986]	novel novel kbp	transport, extracellular region, cellular_component cell, intracellular, cellular_component
			ENSGMOG0000000007363	1855			kbp	lipid metabolic process, signal transduction
			ENSGMOG0000000007425	883			kbp	chromosome, organelle, cell, intracellular, cellular_component, cellular nitrogen compound metabolic process, DNA metabolic process cell, cellular_component
cgpGmo-S1731			ENSGMOG000000002678	1823		glucose-6-phosphatase, catalytic subunit [Source:HGNC Symbol;Acc:4056] leucine rich repeat containing 26 [Source:HGNC Symbol;Acc:31409] RNA binding motif protein 45 [Source:HGNC Symbol;Acc:24468]	kbp kbp kbp	
cgpGmo-S1406			ENSGMOG000000002301	0	intron		kbp	
Gm0588_0274			ENSGMOG000000002427	3454			kbp	
Gm335_0159			ENSGMOG000000002449 ENSGMOG000000009136	821 0	S	cytochrome c, somatic [Source:HGNC Symbol;Acc:19986] ribosomal protein L3 [Source:HGNC Symbol;Acc:10332]	kbp kbp	ribosome, translation, organelle, biosynthetic process, cytoplasm, cell, intracellular, cellular_component
			ENSGMOG000000009149	3653		solute carrier family 25, member 39 [Source:HGNC Symbol;Acc:24279]	kbp	

Table S4. Ranked list of the MGI GOslim2 summary categories represented in the GO-terms associated with gene models located within 10kb up- or downstream from outlier loci (listed in Table S3)

GO Class ID	Definitions	Counts	Fractions
GO:0008152	metabolism	48	23.08%
GO:0007275	developmental processes	24	11.54%
GO:0006810	transport	20	9.62%
GO:0003676	nucleic acid binding	16	7.69%
GO:0007165	signal transduction	14	6.73%
GO:0019538	protein metabolism	12	5.77%
GO:0016301	kinase activity	10	4.81%
GO:0005215	transporter activity	10	4.81%
GO:0016020	membrane	8	3.85%
GO:0004871	signal transducer activity	8	3.85%
GO:0030234	enzyme regulator activity	8	3.85%
GO:0005886	plasma membrane	6	2.88%
GO:0005634	nucleus	4	1.92%
GO:0006259	DNA metabolism	3	1.44%
GO:0016070	RNA metabolism	3	1.44%
GO:0005840	ribosome	2	0.96%
GO:0005929	cilium	2	0.96%
GO:0006950	stress response	2	0.96%
GO:0016265	death	2	0.96%
GO:0005576	extracellular	2	0.96%
GO:0005739	mitochondrion	2	0.96%
GO:0007155	cell adhesion	2	0.96%
Total		208*	100.00%

* 56 single occurrence GO-terms did not fit any GOslim2 category

Table S5. Pairwise differentiation between samples based on all loci (n=160). Cells above the diagonal show pairwise FST and cells below the diagonal show results from pairwise chi-square tests for significant differences in allele frequencies. * indicates p<0.05, ** indicates significance after FDR correction (q<0.05)

		4T								4VsW		3NO				3Ps		
		1928	1960	1968	1974	1976	1983	2002	2008	1960	2010	1960	1973	1990	2010	1964	1993	2010
4T	1928	-	0.074	0.068	0.052	0.071	0.062	0.086	0.066	0.083	0.063	0.063	0.077	0.074	0.067	0.078	0.072	0.075
	1960	**	-	-0.002	-0.007	0.004	0.017	0.001	0.002	0.014	0.016	0.005	0.000	0.007	0.000	0.004	0.000	0.001
	1968	**		-	-0.003	-0.001	0.005	0.003	-0.002	0.010	0.006	0.005	0.004	0.016	0.007	0.010	0.008	0.000
	1974	**			-	0.000	0.009	-0.002	-0.002	0.005	0.006	-0.001	-0.004	0.004	0.000	0.002	-0.004	-0.005
	1976	**	*			-	0.006	0.000	0.000	0.003	0.006	0.016	0.003	0.026	0.014	0.019	0.016	0.000
	1983	**	**	*	*	*	-	0.013	0.006	0.013	0.000	0.020	0.016	0.034	0.021	0.026	0.022	0.010
	2002	**					**	-	0.003	0.001	0.015	0.010	0.002	0.023	0.008	0.013	0.011	-0.004
	2008	**	*				**	*	-	0.009	0.006	0.015	0.003	0.020	0.007	0.014	0.012	0.002
4VsW	1960	**	**	*		*	**	*	*	-	0.016	0.022	0.004	0.042	0.020	0.023	0.023	0.008
	2010	**	**	**	*	*	*	**	**	*	-	0.021	0.014	0.027	0.019	0.024	0.019	0.010
3NO	1960	**	*	*		**	**	*	**	**	**	-	0.011	0.013	0.005	0.009	0.001	0.007
	1973	**		*			**	*	*	*	**	*	-	0.015	0.006	0.010	0.007	-0.001
	1990	**	*	**	*	**	**	**	**	**	**	**	**	-	0.001	0.002	0.001	0.014
	2010	**		*		**	**	*	*	**	**	*	*		-	0.002	-0.003	0.002
3Ps	1964	**	*	*		**	**	*	**	**	**	**	*			-	-0.005	0.010
	1993	**		*		**	**	*	**	**	**						-	0.006
	2010	**					**			*	**			**		*		-

Table S6. Pairwise differentiation between samples based on non-outlier loci (n=101). Cells above the diagonal show pairwise Fst and cells below the diagonal show results from pairwise chi-square tests for significant differences in allele frequencies. * indicates p<0.05. No tests were significant after FDR correction (q<0.05).

		4T								4VsW		3NO				3Ps		
		1928	1960	1968	1974	1976	1983	2002	2008	1961	2010	1960	1973	1990	2010	1964	1993	2010
4T	1928	-	0.006	0.005	-0.006	-0.002	0.003	0.009	0.000	-0.007	0.004	0.001	0.001	0.006	0.000	0.012	0.007	0.000
	1960	*	-	-0.007	-0.012	-0.004	-0.001	0.000	-0.002	0.002	0.001	0.001	0.000	-0.003	-0.003	-0.001	-0.001	-0.001
	1968	*		-	-0.007	-0.005	-0.003	0.001	-0.004	0.003	0.002	0.002	0.004	0.000	-0.001	0.001	-0.001	-0.003
	1974				-	-0.006	-0.007	-0.006	-0.005	-0.007	-0.005	-0.006	-0.007	-0.005	-0.004	-0.004	-0.008	-0.010
	1976					-	0.000	0.001	-0.003	0.002	-0.003	0.001	0.004	0.001	0.003	0.004	0.003	-0.003
	1983	*					-	0.000	-0.002	-0.002	0.002	0.000	0.005	0.003	-0.001	0.001	-0.001	-0.006
	2002	*						-	0.005	0.000	0.006	-0.002	0.007	0.004	0.000	0.001	0.001	-0.004
	2008	*							-	0.002	0.000	0.004	0.003	0.002	0.000	0.003	0.002	0.000
4VsW	1961									-	-0.001	0.003	0.002	0.006	-0.004	-0.003	-0.002	-0.001
	2010								*		-	0.009	0.005	0.003	0.005	0.006	0.005	-0.003
3NO	1960									*	*	-	0.004	0.005	-0.001	0.001	-0.006	-0.007
	1973	*											-	0.001	0.002	0.003	0.002	0.003
	1990	*												-	-0.001	0.000	0.002	0.000
	2010														-	0.000	-0.001	-0.002
3Ps	1964	*									*					-	-0.005	0.000
	1993	*															-	-0.004
	2010																	-

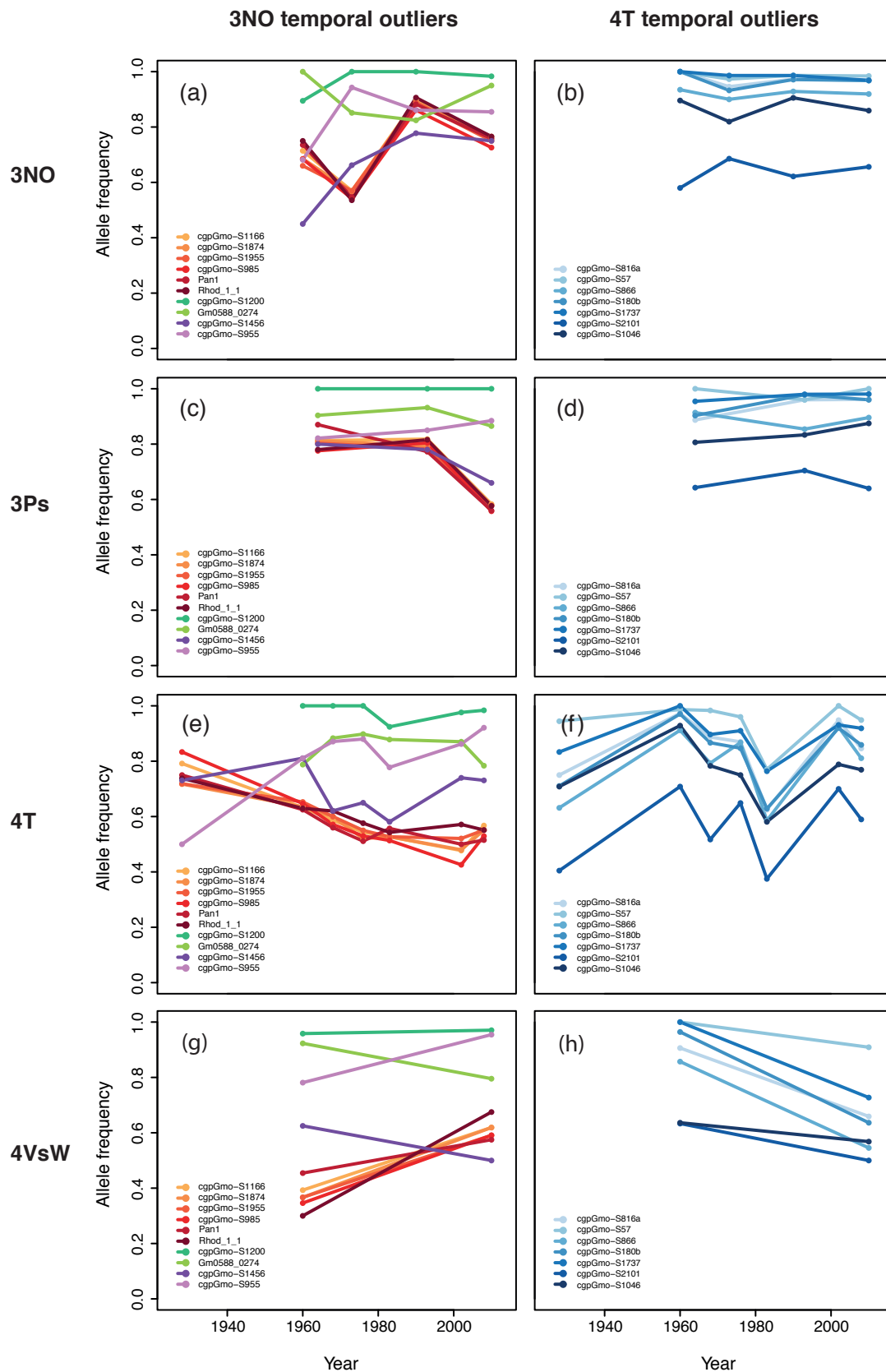


Fig. S1. Observed allele frequencies at significant 3NO temporal outliers (left panel) and significant 4T temporal outliers (right panel) in samples from different years in 3NO (a and b), 3Ps (c and d), 4T (e and f) and 4VsW (g and h). Loci in linkage group 12 are plotted in different shades of blue, loci in linkage group 1 in different shades of red and orange and loci from other linkage groups in green and purple. Dots represent samples and they are connected with lines for easier visualization of temporal trends.

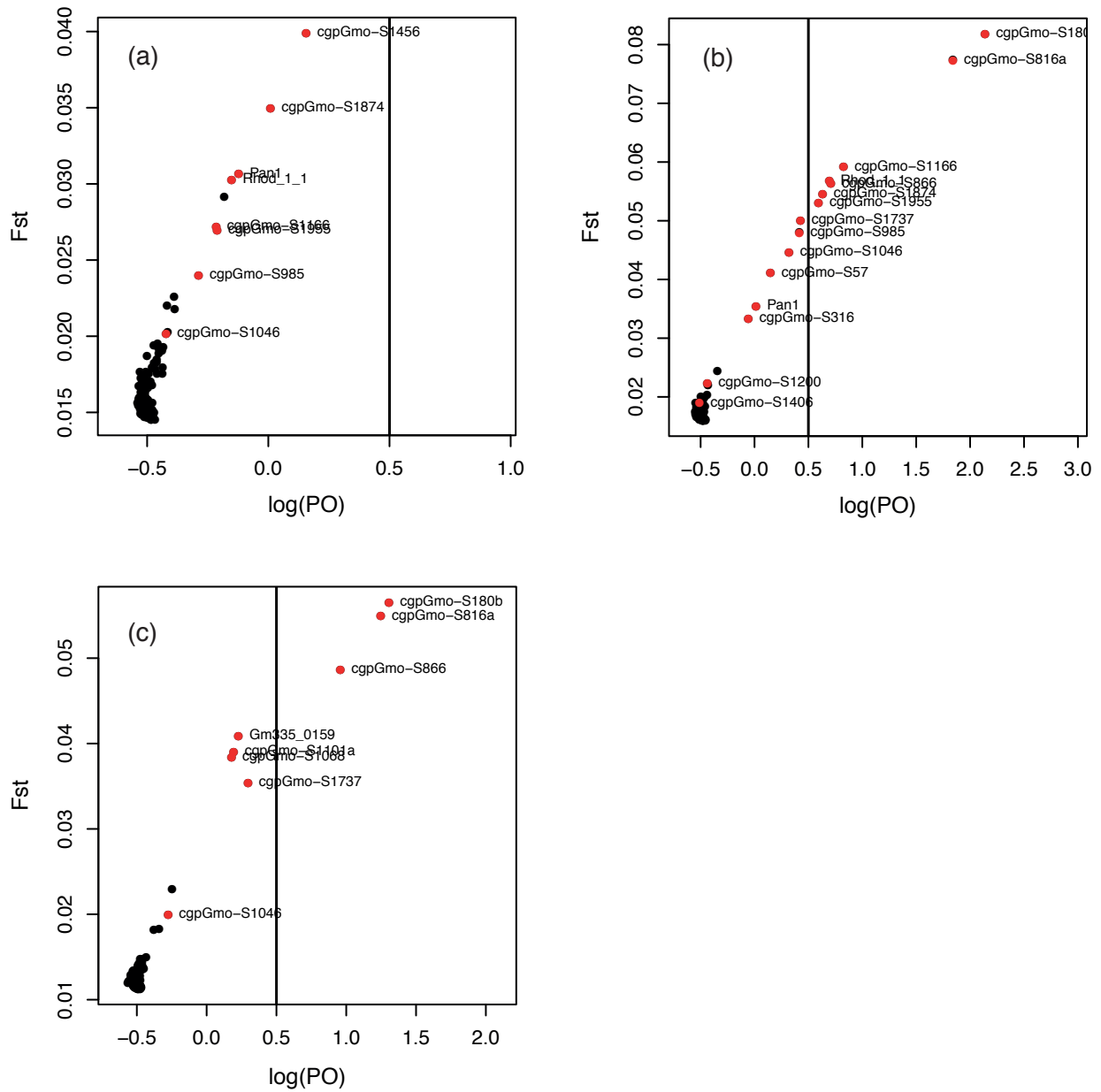


Fig. S2. Results from the BayeScan outlier tests on samples from the 1960s (a), 1980s-1990s (b), and 2000s (c). Each dot represents a locus, illustrating its F_{st} value (y-axis) and \log_{10} of the posterior odds in favor of a model including selection vs. a model of neutral differentiation (x-axis). The vertical lines indicate the significance threshold (controlling the FDR to <0.05) and loci positioned on the right side of these lines are considered outliers. Loci that were also significant in the Lositan analysis are marked in red.

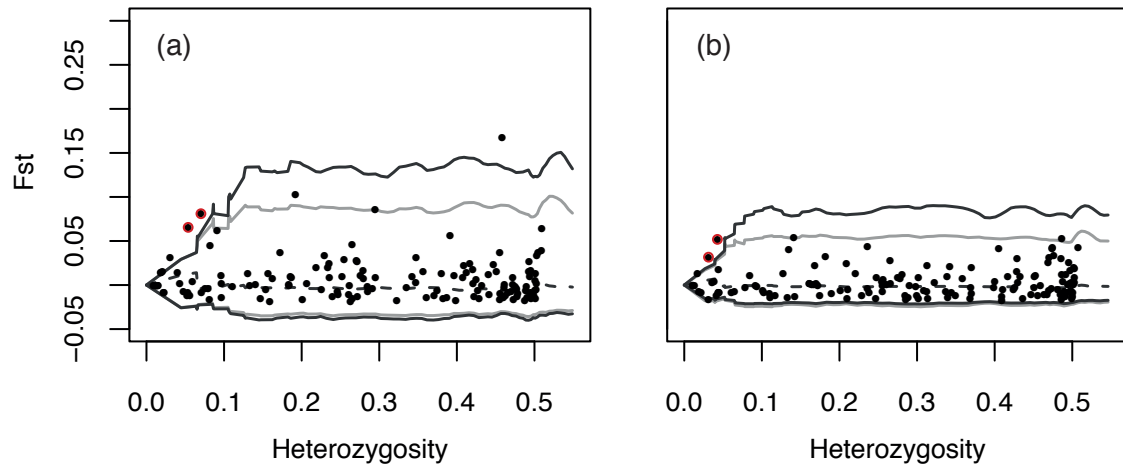


Fig. S3. Results from the Lositan spatial outlier tests in the 1960s (a) and 2000s (b) excluding the 4VsW sample. Each dot represents a locus, illustrating its spatial differentiation (F_{st} , y-axis) against its mean heterozygosity (x-axis). The lines represent the 95% (grey) and the 99% (black) confidence envelopes of the simulated neutral distribution. Significant outliers after FDR correction ($q < 0.05$) are circled in red.

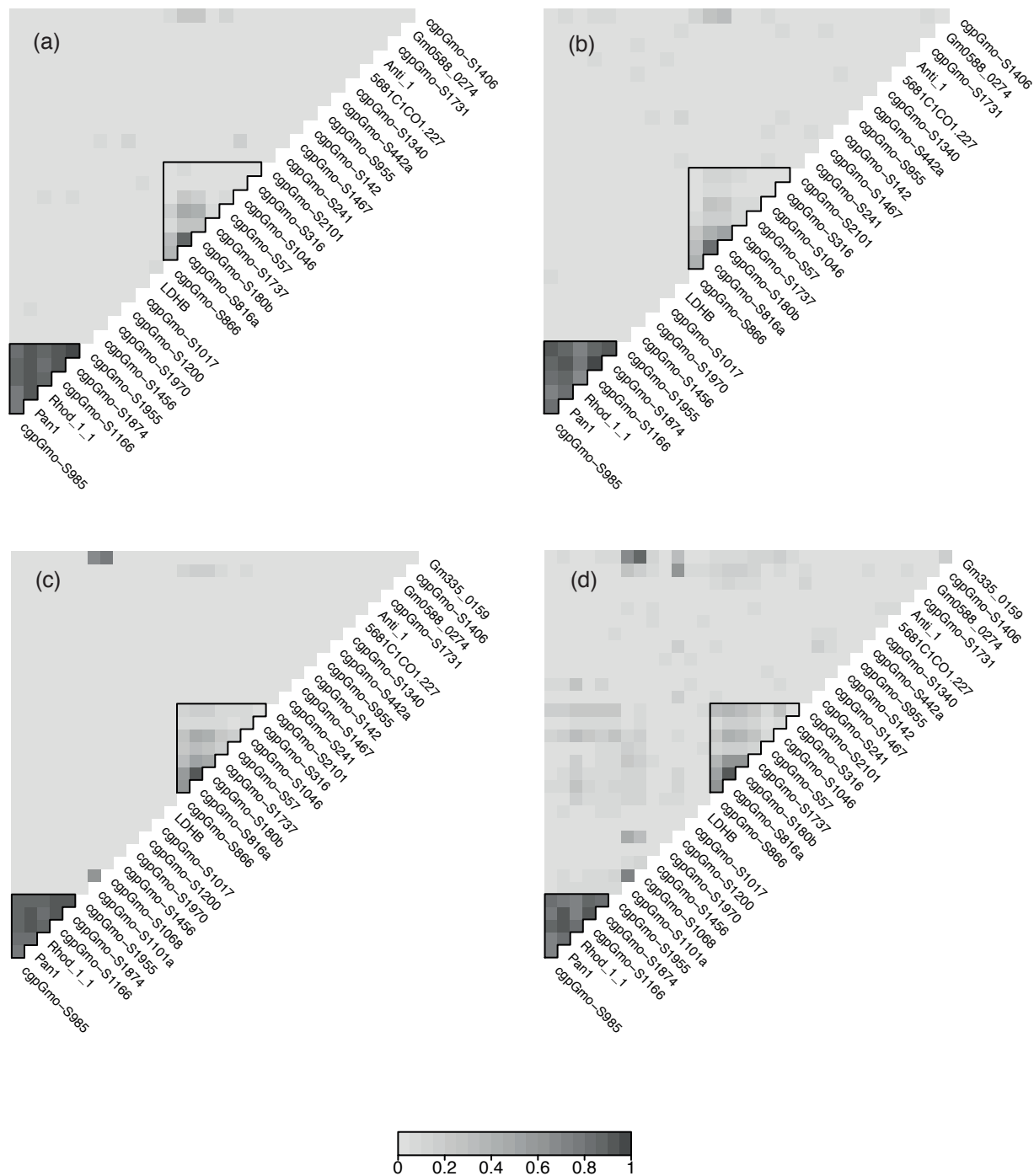


Fig. S4. LD patterns among the temporal and spatial outlier loci in 3NO (a), 3Ps (b), 4T (c), and 4VsW (d). The heatmaps show pairwise r^2 (see scale on plot) between loci computed from all samples (years) pooled within each population. Loci are ordered by linkage group and position within linkage group, where known, in ascending order from left to right. Linkage group 1 and 12 are highlighted.

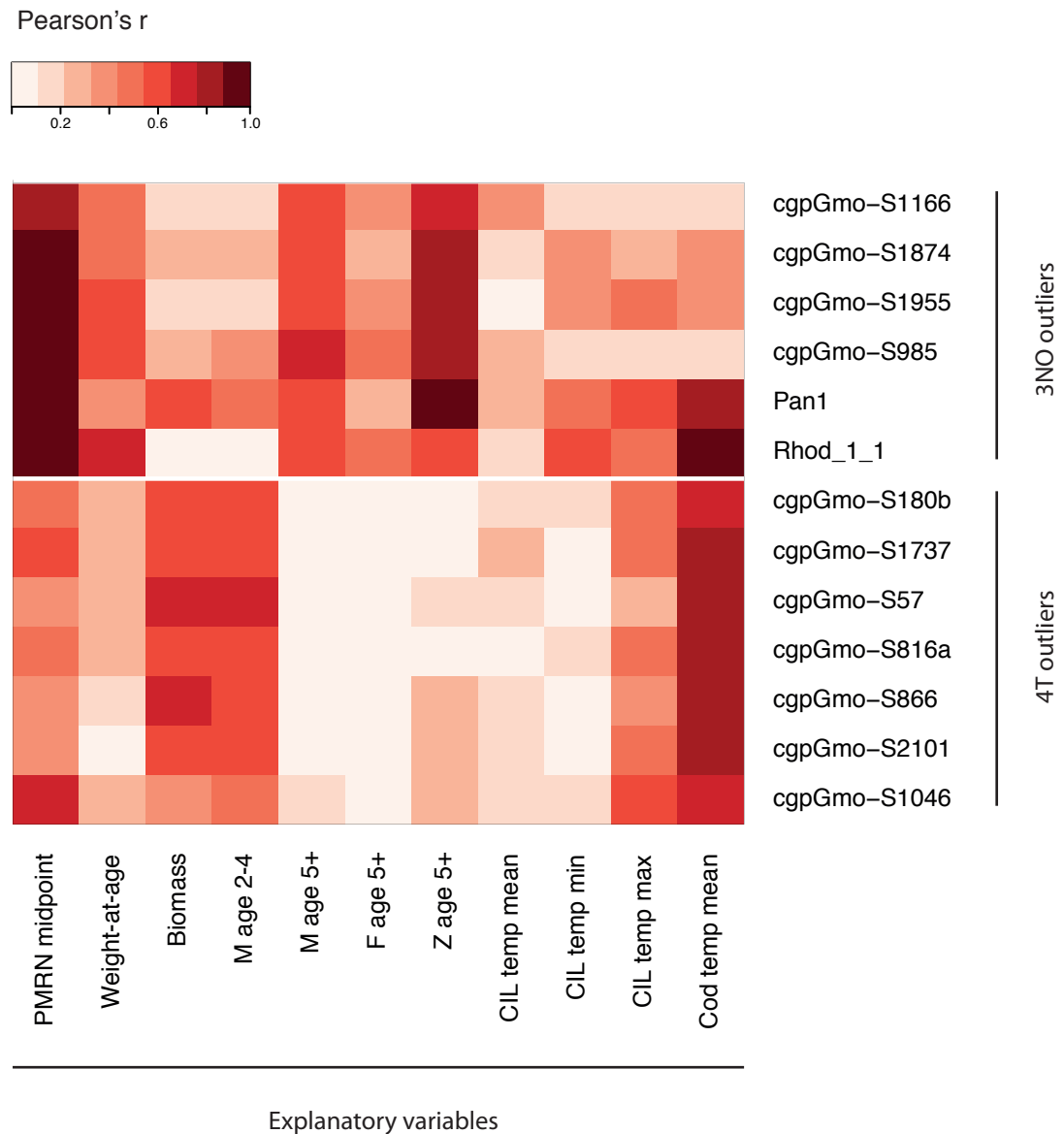


Fig. S5. Heatmap showing Pearson's correlation coefficients (r ; see scale on plot) between temporal patterns in allele frequencies at outlier loci and the selected explanatory variables in the 4T population. The upper and lower panel show loci that initially were identified as temporal outliers in 3NO and 4T, respectively.

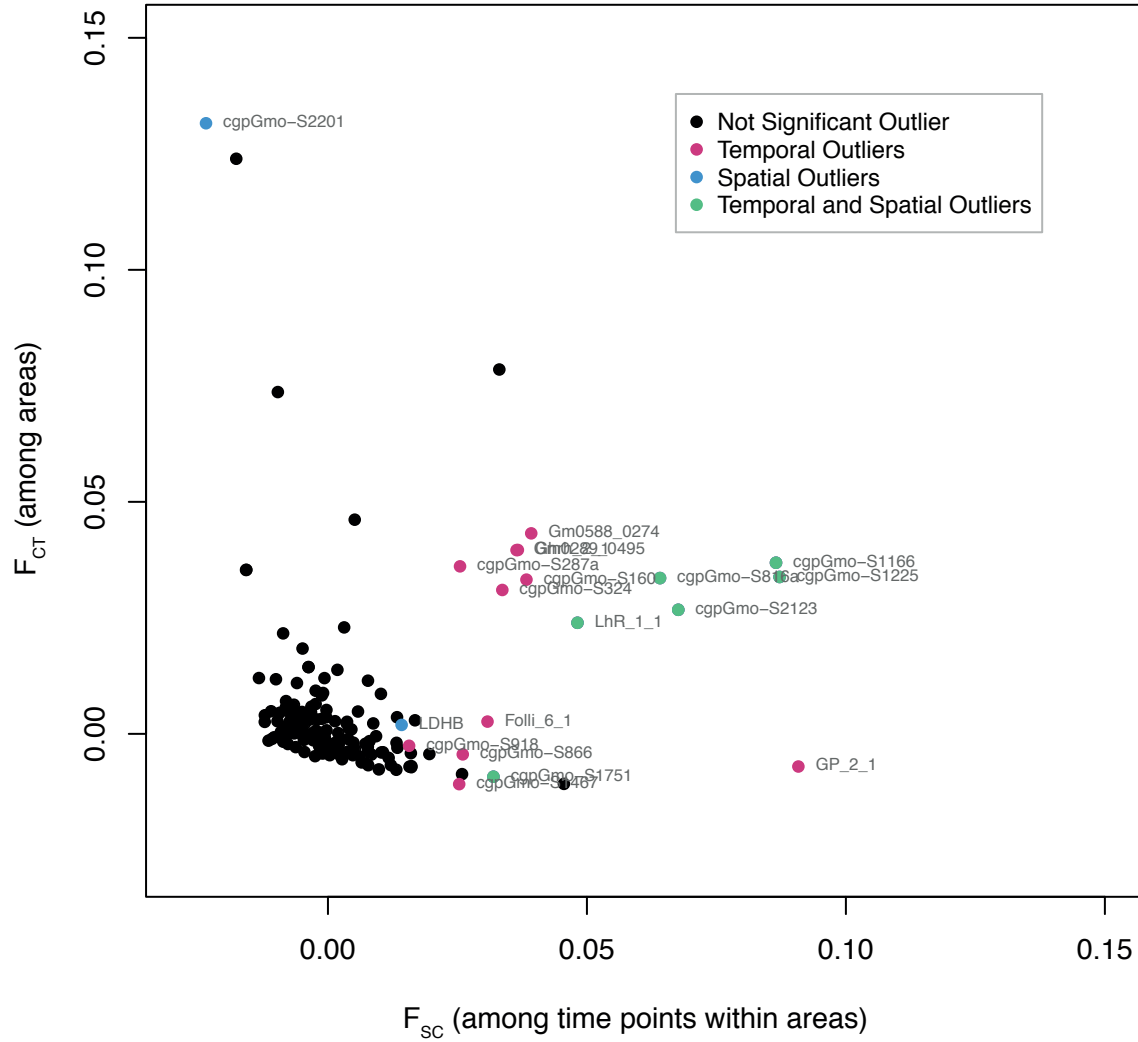


Fig. S6. Results from the locus-specific AMOVA. Each dot represents a locus, illustrating how the variance in observed allele frequencies is partitioned into differences between time points within areas (F_{SC} , x-axis) and differences between areas (F_{CT} , y-axis). Temporal and spatial outlier loci are colored pink and blue, respectively, and loci that are both spatial and temporal outliers are plotted in green.

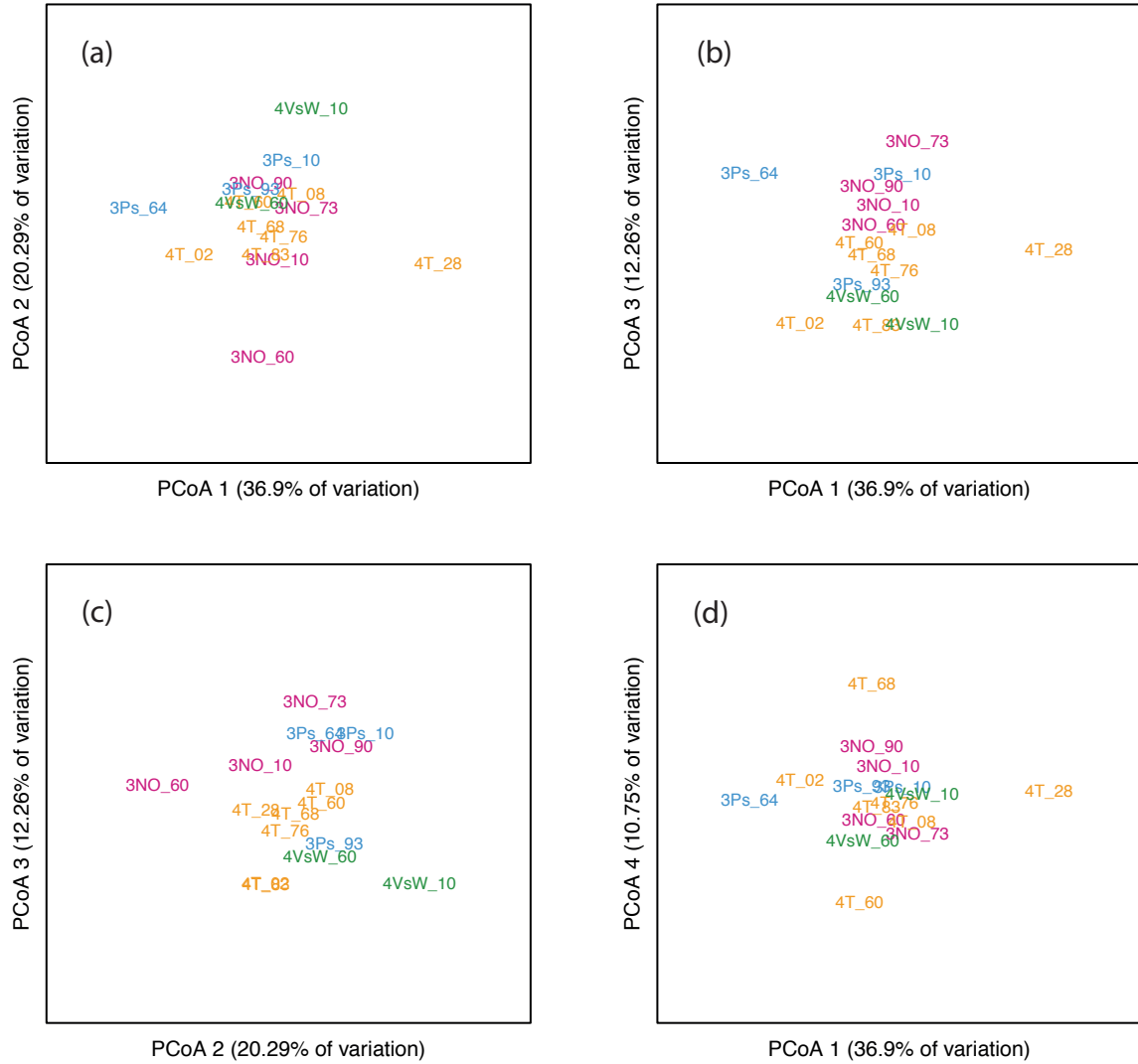


Fig. S7. Sample positions along the primary axes of the PCoA based on pairwise F_{st} matrices computed from non-outlier loci only ($n=101$). Samples from 3NO (pink) seem to cluster together on axes 1 and 3 (b). Samples from 3Ps (blue) cluster together on axes 2 and 4 (c and d). Samples from 4T (yellow) cluster together on axes 2 and 3 (c) and samples from 4VsW (green) on axes 1 and 3 (b).

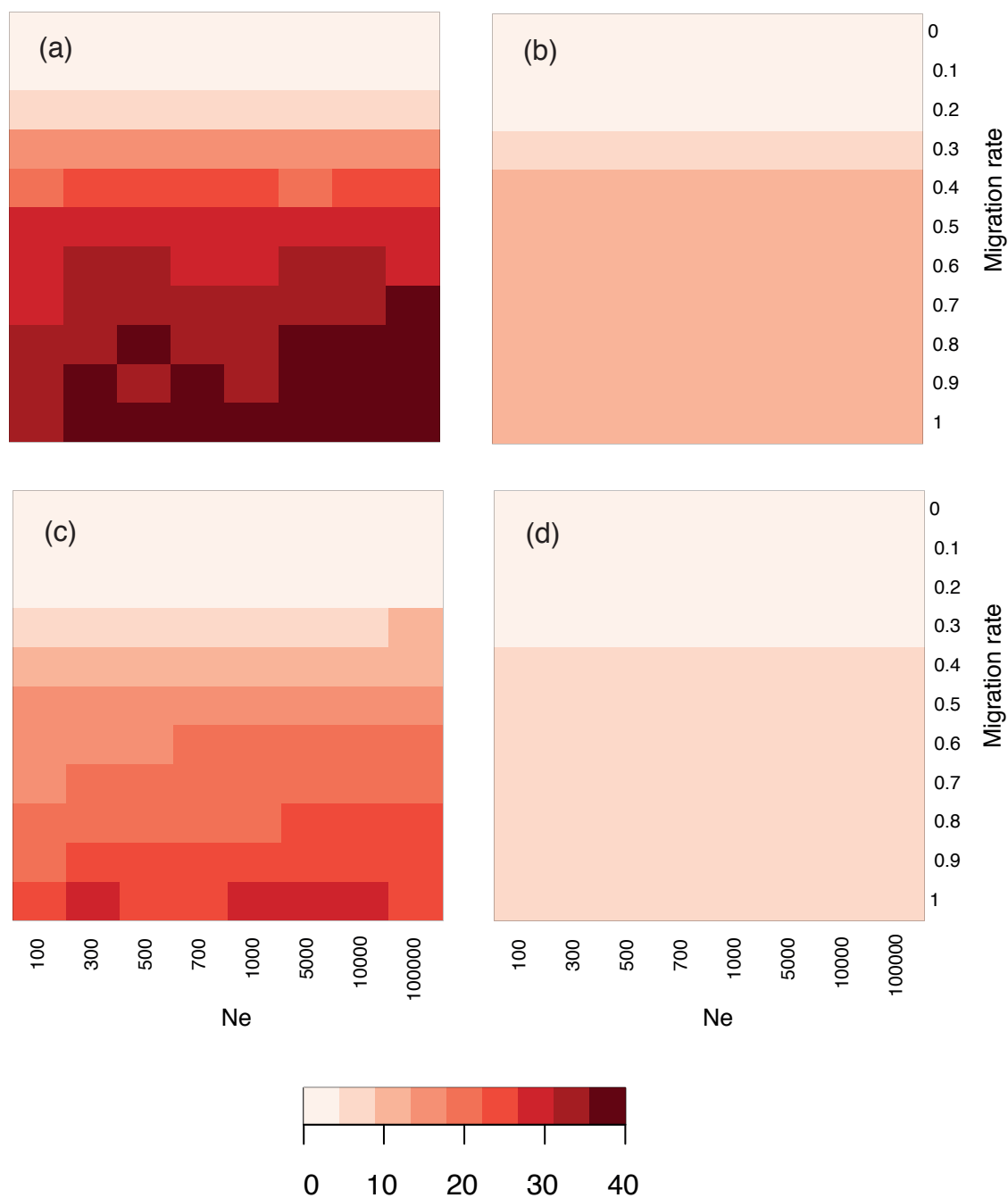


Fig. S8. Heatmaps showing the maximum number of temporal outliers (regardless of locus identity) obtained in simulations of various combinations of local N_e and migration rates from the other populations for focal population 3NO (a), 3Ps (b), 4T (c), and 4VsW (d). See SuppInfo ST3 for details.

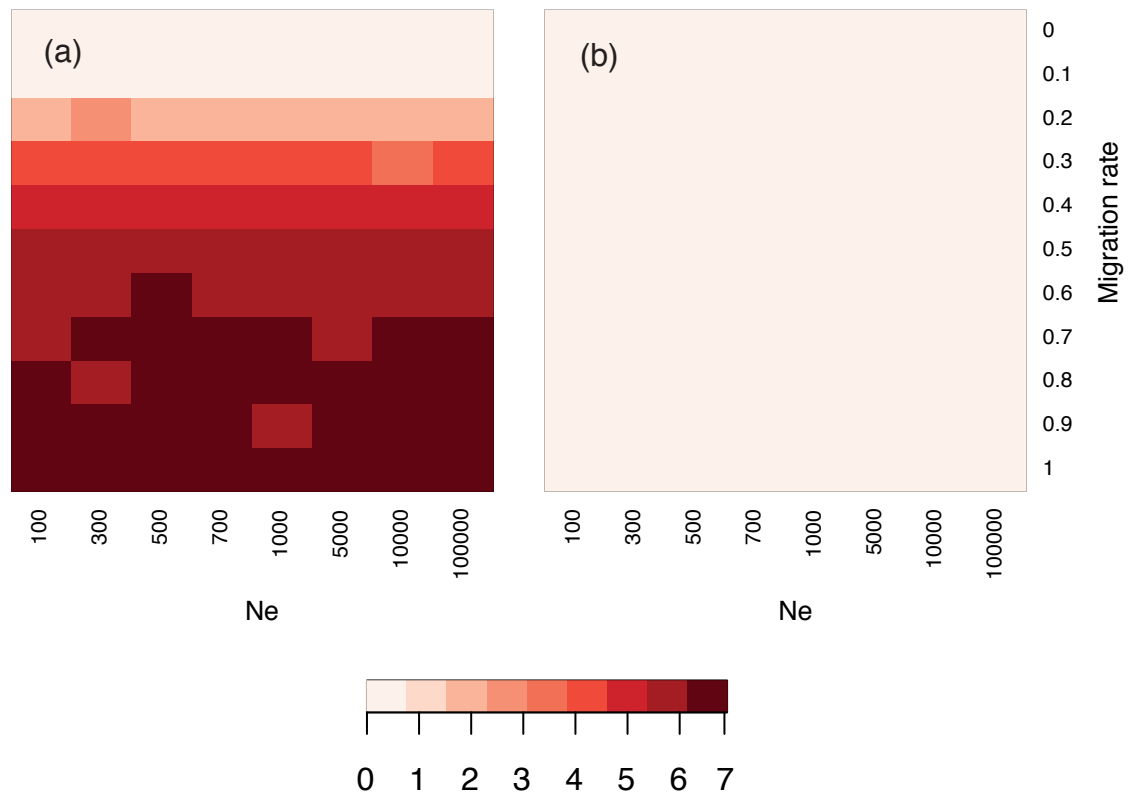


Fig. S9. Heatmaps showing the number of observed temporal outlier loci (in the real data) that also became temporal outliers in simulations of various combinations of local N_e and migration rates from the other populations for focal population 3NO (a) and 4T (b). See SupplInfo ST3 for details. Results are not shown from 3Ps and 4VsW because they had ≤ 1 significant temporal outlier.

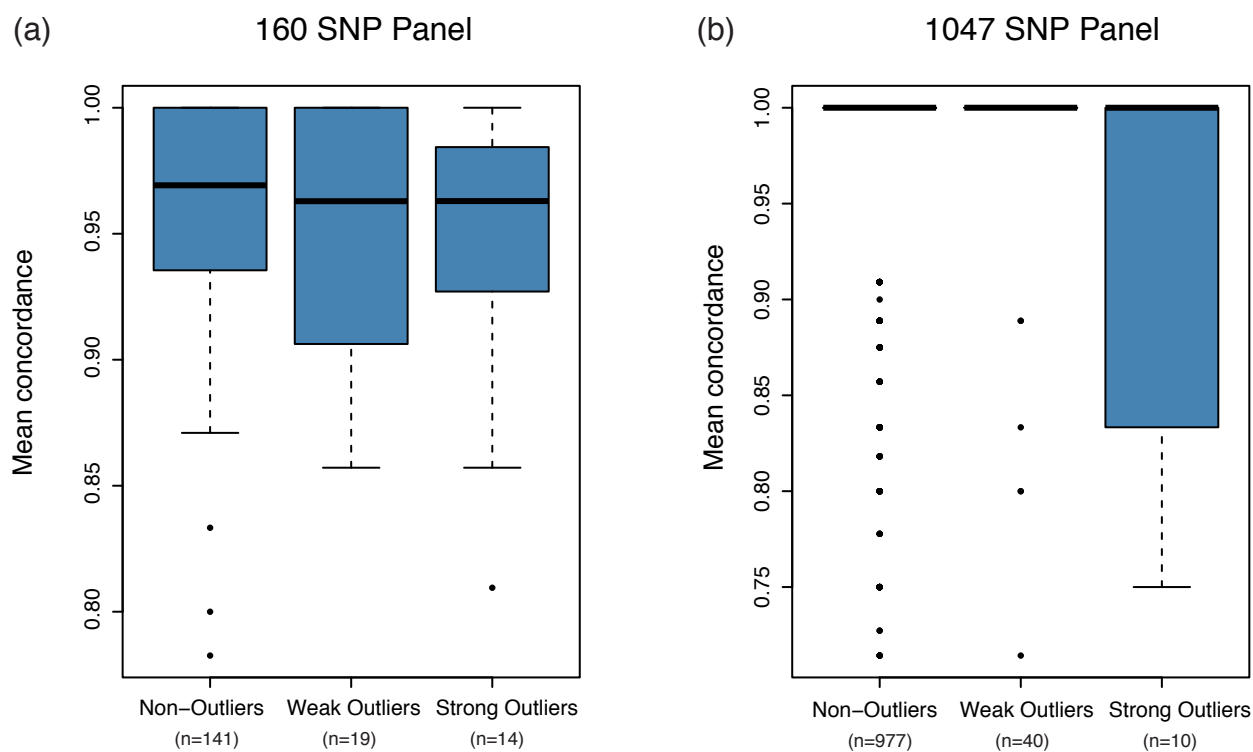


Fig. S10. Comparison of the distribution of genotyping concordance among 29 (a) or 10 (b) replicate samples for SNPs that were not outliers in any comparison (Non-Outliers), SNPs that were 95% outliers but not significant after FDR correction (Weak Outliers) and SNPs that remained significant outliers in either time or space after FDR correction (Strong Outliers). The horizontal band in each box represents the median, the bottom and top of the boxes represent the 25th and 75th percentiles, and the error bars define the 5th and the 95th percentiles. Outlier data points are marked by dots.

CHAPTER 4

Spatiotemporal SNP analysis reveals pronounced biocomplexity at the northern range margin of Atlantic cod *Gadus morhua*

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Abstract

Accurate prediction of species distribution shifts in the face of climate change requires a sound understanding of population diversity and local adaptations. Previous modeling has suggested that global warming will lead to increased abundance of Atlantic cod (*Gadus morhua*) in the ocean around Greenland, but the dynamics of earlier abundance fluctuations are not well understood. We applied a retrospective spatiotemporal population genomics approach to examine the temporal stability of cod population structure in this region and search for signatures of divergent selection over a 78-year period spanning major demographic changes. Analyzing >900 gene-associated SNPs in 847 individuals, we identified four genetically distinct groups that exhibited varying spatial distributions with considerable overlap and mixture. The genetic composition had remained stable over decades at some spawning grounds, whereas complete population replacement was evident at others. Observations of elevated differentiation in certain genomic regions are consistent with adaptive divergence between the groups, indicating that they may respond differently to environmental variation. Significantly increased temporal changes at a subset of loci also suggest that adaptation may be ongoing. These findings illustrate the power of spatiotemporal population genomics for revealing biocomplexity in both space and time and for informing future fisheries management and conservation efforts.

Keywords: Genetic monitoring, temporal change, population structure, adaptive divergence, contemporary evolution, marine fish, climate change, Greenland

INTRODUCTION

The geographical distribution of many plants and animals is expected to shift poleward in face of climate change, as revealed by both modeling and empirical investigations (e.g. Parmesan and Yohe 2003; Burrows et al. 2011; Chen et al. 2011). While studies documenting these trends have provided important insights, they almost exclusively focus on the species level, ignoring that species are made up of populations that each may harbor unique adaptations to specific local environments and therefore will react differently in response to altered conditions (Hilborn et al. 2003; Schindler et al. 2010; Kelly et al. 2011). It is typically unclear to what extent climate-induced species distribution shifts simply reflect the sum of different populations moving to new areas as they each track the changing location of their environmental “niche”. Alternatively, species-level shifts could result from extinction of certain populations—and therefore loss of a unique portion of the species’ evolutionary legacy—coupled with local growth and spatial expansion in previously marginal populations. With changing conditions, rapid adaptation may also be required, both for maintaining current distributions and for colonizing new habitat (Gienapp et al. 2008; Hoffmann and Sgrò 2011). Understanding population diversity, temporal dynamics, adaptive divergence and evolutionary potential is therefore critical for making accurate predictions about the future distribution of biodiversity, both at the species and population levels.

Atlantic cod (*Gadus morhua* L.) in the waters around Greenland offers an exceptional opportunity for studying these issues at a northern range edge, in a habitat that currently appears marginal but is predicted to become much more important for the species with the substantial ocean warming forecasted for the region (Drinkwater 2005). Greenland is likely to be among the most recently colonized parts of the contemporary range of Atlantic cod (Bigg et al. 2008), and historical records show that its abundance here has exhibited episodic extreme fluctuations (Hansen 1949; Buch et al. 1994). The most recent period of high abundance occurred between 1930 and the late 1960s, when the continental shelf off Greenland’s west coast supported an enormous cod fishery that for decades yielded annual landings >250,000 tons (Buch et al. 1994; Horsted 2000). After 1970, however, both the spawning biomass and recruitment declined by nearly 100%, leading to a period of virtual absence of cod from the offshore waters, although they remained present in lower abundance inshore (Horsted 2000; Storr-Paulsen et al. 2004; Rätz and Lloret 2005). Multiple similar abundance outbursts, coupled with varying expansion and retraction of the northern distribution limit, have been reported over the past centuries (Hansen 1949; Buch et al. 1994). These patterns have, at least partly, correlated with ocean temperatures (Buch et al. 1994; Stein 2007), and indeed, coinciding with ocean warming in recent years, increased cod biomass has been observed both inshore and offshore in Greenland (Drinkwater 2009; ICES 2011).

Yet, it remains unclear if increases in abundance—now and in the past—are caused by recurrent colonization by populations from elsewhere or if they result from sudden growth in resident populations that have maintained a stable distribution through periods of low abundance. Tagging studies and egg distribution surveys have suggested that there are separate inshore and offshore spawning components within Greenland and that inflow of eggs and larvae from Icelandic waters also makes an important contribution to local recruitment (Buch et al. 1994; Storr-Paulsen et al. 2004). A recent study demonstrated genetic differentiation between samples of cod collected offshore and inshore during the feeding season (Pampoulie et al. 2011), but this did not clarify the spatial genetic population structure of reproductively isolated units or how the different components have been distributed over time. It also did not comprehensively assess adaptive divergence between the groups, including their ability to rapidly adapt to changing conditions.

With recent improvements in high-throughput genotyping methods, it is now becoming possible to screen large panels of genetic markers, even in studies of non-model organisms such as Atlantic cod. The increased genomic coverage provides unprecedented opportunities, both for detecting genetic variation that is affected by selection and for resolving weak population structure, as

characterize many marine fish. When these methods can be applied to historical DNA samples, which provide unique insights into the past, we obtain an extremely powerful tool for tracking population structure and adaptive divergence in both space and time.

We here illustrate this approach with one of the first spatiotemporal population genomics studies on wild populations published for any species. Utilizing invaluable collections of archived material, the study is based on extensive sampling of Greenlandic spawning grounds both contemporarily and during the historical period of maximum abundance 5-8 decades ago. Through analysis of >900 single nucleotide polymorphisms (SNPs), we disentangle locus-specific from genome-wide patterns of variation in both space and time to shed light on 1) how many separate cod populations inhabit Greenlandic waters; 2) how stable the population structure and the distribution of the different components has been over time; 3) whether the populations are adapted to different environmental conditions; and 4) whether we can observe signatures of ongoing adaptation over the study period. Elucidating these questions will provide important insights about the ongoing colonization of northern habitats and help improve predictions of future changes.

MATERIALS AND METHODS

Samples

Contemporary samples of fin tissue were collected from 13 known spawning areas in Greenland during the spawning seasons (March-May) of 2008 and 2010 (Fig 1). Where available, we matched these samples with historical otoliths collected in the same locations during the spawning season 55-80 years ago. The otoliths had been archived individually in paper envelopes at room temperature at the Greenland Institute of Natural Resources. Since low abundance of cod on the west coast banks in recent years prevented extensive contemporary sampling here, we added additional historical samples from this area. For reference, we supplemented the data with three population samples from different spawning components in Iceland collected in 2002, and a single population sample collected in Greenland during the feeding season in 2005 (previously analyzed by Nielsen et al. 2012). All sampled individuals were of reproductive age and most were in spawning condition. Sample sizes as well as sampling locations and years are listed in Table 1.

Molecular analysis and genotyping

DNA was extracted with Omega EZNA Tissue DNA kits (Omega Bio-Tek, USA) following the manufacturer's instructions for fresh tissue and the procedure described by Therkildsen et al. (2010a) for otoliths. To minimize contamination risk, all DNA extraction and PCR preparation from otoliths were conducted in an ancient DNA laboratory where no contemporary samples had been processed. We also pre-screened the historical extracts by amplifying four highly polymorphic microsatellites (mean number of alleles 19) and discarding individuals that showed evidence of cross-sample contamination (amplification of >2 alleles for any locus) or that failed to produce reliable amplification within 2-3 attempts.

Samples that passed the pre-screening were genotyped for 1152 previously validated transcriptome-derived SNPs (Moen et al. 2008; Nielsen et al. 2009; Hubert et al. 2010; Bowman et al. 2011; Hemmer-Hansen et al. 2011). Of these SNPs, 766 were already positioned on the published linkage map for Atlantic cod (Borza et al. 2010; Hubert et al. 2010). By mapping 120 bp of the flanking sequence surrounding each SNP on to scaffolds of ATL COD1A build of the cod genome (Star et al. 2011) using BLASTN with an e-value threshold of $1e-10$, 133 additional SNPs

could be anchored (for a total of 899 of the SNPs) to linkage groups (LGs). The applied SNP panel was fairly evenly distributed among the 23 LGs (between 20 and 60 (mean of 39) SNPs per LG), ensuring broad genomic coverage.

The SNP genotyping was performed at the Roslin Institute at the University of Edinburgh, Scotland, using the Illumina GoldenGate platform following the manufacturer's protocol. This array-based technology is based on hybridization of short (<60 bp) locus- and allele-specific probes to the template DNA and should therefore be well suited for fragmented historical DNA. To minimize the risk of cross-sample contamination, historical and contemporary samples were kept separate during all steps. The SNP data were visualized and analyzed with the GenomeStudio Data Analysis software package (Illumina Inc.). All genotype cluster positions were edited manually and we only included data points with GenCall score >0.25 and samples and SNPs with a call rate >0.7.

Data quality control and summary statistics

To evaluate the reproducibility of genotype calls, 26 historical DNA extracts were analyzed in two independent assays and a single control individual was included on all 96-well plates. We computed the genotyping concordance for each SNP as the number of identical genotype calls divided by number of samples where both replicates had been successfully genotyped. SNPs with a mean concordance <0.9 were discarded from the data set.

We computed expected and observed heterozygosity (H_e and H_{obs}) and tested for Hardy-Weinberg equilibrium (HWE) in all samples using 10^5 permutations with the Monte Carlo procedure implemented in the R-package ADEGENET (Jombart 2008). The degree of linkage disequilibrium (LD) between all pairs of loci within each sample was evaluated with the GENETICS package for R (Warnes 2003). Here, and where appropriate throughout the analysis, we corrected for multiple testing by computing the expected false discovery rate (FDR), or q-value, for each test based on the distribution of p-values using the R-package QVALUE (Storey and Tibshirani 2003). We considered tests significant when the FDR was <5% ($q < 0.05$).

Population structure

To examine the patterns and levels of differentiation among samples, we computed pairwise F_{ST} between all samples with the Fstat function from the GENELAND package in R (Guillot et al. 2005) and tested for pairwise differences in allele frequencies among all samples using chi-square tests, as implemented in the software CHIFISH (Ryman 2006).

For further exploration of the population structure, we applied discriminant analysis of principal components (DAPC; Jombart et al. 2010) as implemented in R package ADEGENET (Jombart 2008). Since we did not *a priori* know how many populations were represented by our data, we first used the find.clusters function to run successive K-means clustering of the individuals for $K=1:20$, and identified the best supported number of clusters through comparison of the Bayesian Information Criterion (BIC) for the different values of K. We then applied the dapc function to describe the relationship between these inferred groups. This function constructs synthetic variables, discriminant functions (DFs), that maximize variation *between* while minimizing variation *within* groups and computes coordinates along these functions for each individual. To avoid over-fitting, we retained only the 111 first principle components (PCs) from the preliminary data transformation step (indicated to be the optimal number based on the optim.a.score function), representing 46% of the total variation in the data set (analysis including all PCs yielded virtually identical results).

Based on the derived DFs, we obtained posterior cluster membership probabilities for each individual and computed the mean membership probability to the different clusters for each

sample. We then categorized samples with mean membership probability of >0.6 to one of the clusters as “pure” samples and the others as “mixed” samples (for use in the outlier tests and LD analyses). To cross-validate the robustness of cluster assignments, we randomly selected half the individuals from each sample as our training data and the other half as our hold-out data. We then re-computed the clustering and DAPC analysis based on the training data alone and applied the `predict.dapc` function to position the “hold-out” individuals onto these new DFs. This way, posterior membership probabilities for the hold-out individuals reflected how reliably individuals that had not been used to define DFs would assign to clusters.

To assess how much of the observed structure was driven by loci under selection, we repeated all the analysis with a subset of the data excluding loci that were spatial or temporal outliers (see below) or exhibited high LD (mean $r^2 > 0.1$ within “pure” samples) with other loci.

Spatial outlier detection

To identify loci that showed divergent patterns of differentiation compared to neutral expectations and therefore potentially have been affected by selection, we applied the Bayesian approach of Beaumont and Balding (2004) as implemented in the software BAYESCAN 2.1 (Foll and Gaggiotti 2008). We set the prior odds for a model without selection to 10:1 and ran the program with 20 pilot runs of each 5000 samplings followed by an additional burn-in of 50000 and 5000 samplings with a thinning interval of 10. Correcting for multiple testing, the program computes q-values based on the posterior probability for each locus, and we considered loci with $q < 0.05$ in three independent runs significant outliers.

Because hierarchical structuring, as observed in our data, can lead to an excess of false positives if not accounted for in outlier tests (Excoffier et al. 2009), we supplemented the BAYESCAN results with simulations under the hierarchical fdist model as implemented in ARLEQUIN 3.5 (Excoffier and Lischer 2010). For each run, we used 50000 simulation iterations with a null model with 10 groups, each containing 100 demes, and computed q-values based on the derived p-values to consider loci with $q < 0.05$ significant outliers.

For both outlier detection methods, we conducted a series of tests with different subsets of the samples. Initially, we examined the overall patterns with tests including all samples at two cross-sections of time, contemporary and historical, here basing the groupings for the hierarchical model on the cluster of maximum membership probability for each sample (Table 1). Because we were particularly interested in loci under selection between the clusters, we followed up with pairwise comparisons of the clusters (here only including the “pure” contemporary samples) and non-hierarchical tests among the samples within each cluster (historical and contemporary separately).

Temporal outlier detection

We also applied outlier tests to assess whether any loci showed greater temporal differentiation than expected under drift and sampling error alone within the locations where the cluster membership of individuals was relatively stable over time. Because the outlier tests applied above rely on models of spatial variation between multiple populations, they are not directly suitable for examining variation over time within a single population. We therefore adapted the fdist approach (used for the hierarchical spatial tests), so that it would better fit a temporal scenario (see Supporting information for details). The key difference was that we here generated the neutral expectation through simulations under a Wright-Fisher model of drift over time within a single

population rather than as drift-migration equilibrium between multiple populations. Otherwise, the outlier detection was conducted as in the original approach (Beaumont and Nichols 1996).

A required input parameter for the temporal null model was the number of generations between samples, which we estimated to be between 11-15 in the different locations based on demographic data (see Supporting information). A second required input was the effective size (N_e) of the sampled population, which we estimated for each location based on the temporal variance in allele frequencies between sampling points and which appeared high at all locations (lower 95% confidence limit consistently ≥ 450 , see Supporting information). For each run, we simulated 10^5 loci and computed p-values for each observed locus indicating the probability that it showed greater temporal differentiation than expected from the null model. The temporal outlier analyses were completed with custom R-scripts available upon request.

Environmental correlations

To gain insights about what factors may drive selection in this system, we tested for associations between the spatial distribution of allele frequencies and a range of environmental and seascape parameters. For this analysis, we used the method implemented in the software BAYENV (Coop et al. 2010), which accounts for the underlying population structure when testing for locus-specific environmental correlations in a Bayesian framework. The first step is to estimate a covariance matrix from a set of presumably neutral SNPs. Based on this matrix, the program then computes a Bayes factor (BF) for each locus, reflecting the ratio of posterior support for a model with a linear correlation between an environmental variable and allele frequencies versus a model including the covariance matrix only. Analyzing the historical and contemporary samples separately, we estimated the covariance matrices from a subset of SNPs ($n=618$) excluding outliers and loci in strong LD and used the mean of the two final matrices obtained in two independent runs of each 10^5 iterations of the MCMC chain. We considered locus-environment combinations with a $\log_{10}(\text{BF}) > 1.5$ significant ("very strong evidence" according to Jeffreys (1939) scale).

Environmental data were primarily obtained from the Nucleus for European Modeling of the Ocean (NEMO) shelf sea model. To obtain data that reflected long-term conditions at the sampling locations, we used averages of annual values for 1948-2011 within 7x7 km grid cells. For some of the coastal positions that fell just outside the geographic coverage of the model, data were interpolated from the adjacent grid cells. The Disko Bay (ILL samples) and the Nuuk area were not covered in the model. For Disko Bay, adequate observational data were not available, but for Nuuk, data on certain variables were compiled from historical CTD data downloaded from the ICES Oceans database (<http://ocean.ices.dk/>) and retrieved from archived logbooks (Hedeholm unpublished). We reduced the full set of variables initially considered (Table S1, Supporting information) to a subset including only relatively uncorrelated variables ($\rho < 0.8$, Spearman Rank Correlation Test). The variables considered in the final analysis were latitude, longitude, distance to nearest coastline, annual maximum, mean and range for bottom spring temperature, annual mean, minimum and range for surface spring temperature, and annual mean bottom salinity.

RESULTS

Data quality and summary statistics

DNA extracts from a total of 847 individuals were analyzed with the SNP assay (231 historical samples were discarded due to contamination or poor DNA quality). In these samples, 1011 SNPs

were successfully genotyped; 935 of these passed the quality criteria and were used for analysis. The mean genotype concordance among replicate samples was 98% and the mean call rate for samples was 93%. The different samples were polymorphic for between 86 and 99% of loci and H_e ranged from 0.25-0.32 (Table 1).

In single-locus tests for HWE, 1471 tests (out of 28050) had $p < 0.05$, with the highest concentration in the samples OWE10 and QAQ08 (with 87 and 77 out of 935 loci having $p < 0.05$, respectively). However, after FDR correction, only 13 tests remained significant ($q < 0.05$) and these were distributed among loci and samples. LD analysis revealed variable numbers of significant associations among loci in the different samples, but 1747 of the 436612 possible pairwise comparisons among loci had a mean $r^2 > 0.1$ within “pure” samples (see below). When discarding one locus from each of these LD pairs, 693 loci remained.

Population structure

Pairwise F_{ST} estimates ranged from -0.003 to 0.072 and were highest between ISC02 and most other samples, except the other Icelandic and the Nuuk inshore samples (Fig S1). The majority of pairwise comparisons (336 of 378) showed significant differences in allele frequencies between samples after correction for multiple testing. Notable exceptions were among the Nuuk samples, among the northern west coast Greenland samples, and among the west coast offshore samples (Fig. S1, Supporting information).

Consistent with these results, the K-means analysis revealed that clustering solutions with either three or four groups generated the lowest BIC-scores and therefore were best supported (Fig. S2a, Supporting information). Two groups were consistent in both clustering solutions: one (the “East” cluster) containing the majority of individuals in the Icelandic offshore sample, the east Greenland samples and the southernmost offshore samples from western Greenland, and another (the “West” cluster) containing the majority of individuals from the remaining western Greenlandic samples except the fjord samples from around Nuuk and portions of the contemporary Sisimiut samples (Table 1). The three-cluster solution grouped Icelandic and Nuuk inshore samples together, whereas the four-cluster solution separated these groups (Fig. S3a, Supporting information). Since this separation is geographically meaningful and there is temporally stable significant differences between the samples, we proceeded with the four-cluster solution.

The samples exhibited considerable overlap between the positions of individuals on the DFs. However, when examining the mean coordinates of each sample, it is evident that the first DF (representing 61.7% of the discriminating power) resolves a continuum from the Greenlandic inshore through offshore West and East to Icelandic inshore (Fig. 2). The second DF (representing 27.6% of the discriminating power) separates inshore samples (in both Greenland and Icelandic waters) from offshore samples (Fig. 2a). The third function (representing 10.6% of the power) separates both the inshore and offshore groups into Icelandic and Greenlandic components, except from a few Greenlandic samples that cluster with the Icelandic samples, likely representing migrants (see below; Fig. 2b). Recoding of the coordinates on the first two DFs into signal intensity of red and green color, respectively, provides visualization of the geographic distribution of these patterns (Fig. 1). Inspection of the allele loadings on the DFs revealed that a large number of SNPs spread across different LGs drove the discrimination of the first and the third function, whereas the strongest allele contributions to DF 2 (that separated inshore from offshore) were almost exclusively dominated by SNPs in LG1 (Fig. S4, Supporting information).

With K-means clustering based on the full dataset, 87% of individuals showed posterior membership probability of > 0.95 to one of the four clusters. In the cross-validation where only half of the individuals were used as training data, the assignment power remained high, with 82% of

the hold-out individuals showing posterior membership probability of >0.95 to one of the clusters and 94% of these assigning to the same cluster as in the full data analysis. The consistent results obtained when hold-out individuals were not used for defining clusters or DFs indicate that the reported cluster configuration was well supported by the data.

At the aggregate level, 20 of the 28 samples had mean membership probability >0.6 to a single clusters, while the remaining 8 appeared to consist of mixtures of cod from different clusters (Table 1). Both “pure” and “mixed” samples were primarily made up of individuals that assigned with high probability to a single cluster (Fig. 3). However, some individuals appear to be admixed, showing relatively even membership probabilities between different clusters. Of particular note, the majority of the Greenlandic west coast offshore samples appeared to contain approximately even mixtures of fish with high assignment probability to the “East” and the “West” clusters, respectively. Meanwhile, a vast majority fish in the coastal west coast samples assigned to the “West” cluster (Fig. 3). Two exceptions to this were the contemporary samples from SIS that appeared to contain a considerable proportion of fish assigning to the “Nuuk” cluster, and contemporary samples from PAA and QAQ that appeared to be made up of fish from the “Iceland-inshore” and the “East” cluster, respectively (Fig. 3). Since the historical samples from these latter two locations contained almost exclusively “West” individuals, the contemporary dominance of the alternate clusters suggests complete population replacement in this area. In contrast to these stark temporal changes, other locations (UMM, ILL, KAP and DAB) exhibited a high degree of temporal stability, as evident both from assignment results (Fig. 3) and from the tight clustering of temporal replicates (Fig. 1 and 2).

When loci potentially under selection (see below) and loci in strong LD were removed from the data, the pairwise F_{ST} coefficients were considerably lower than with all loci (ranging from -0.003 to 0.028), but 259 of 378 comparisons still showed significant differences in allele frequencies (Fig. S1, Supporting information). The K-means clustering clearly indicated that with this data subset, a solution with only two clusters was best supported (Fig. S2b, Supporting information): One cluster containing the Icelandic (both inshore and offshore), the east coast, the contemporary QAQ and PAA as well as portions of the Nuuk samples, and a second cluster containing the remainder of the Greenlandic samples (not a single Icelandic individual assigned to this cluster). The three-cluster solution corroborated this, except that it split the “Nuuk” samples into their own cluster (Fig. S3b, Supporting information).

Spatial outlier detection

In all analyses, BAYESCAN detected considerably more outliers than ARLEQUIN (often more than twice as many), but ARLEQUIN outliers were almost exclusively a subset of BAYESCAN outliers. Here, we present only results on outliers identified by both methods. In the comparison of all contemporary samples, 47 loci were either F_{ST} (differentiation between all samples) or F_{CT} (differentiation between clusters) outliers (the majority both; Table S2, Supporting information), and all but 6 of these loci were located in three high-LD regions of LG1, 2, and 7, respectively. Analysis of the Icelandic samples alone identified a large proportion of the global outliers in LG1 and LG7, but notably not LG2. Within Greenland, the majority of global outliers from LG1 along with a number of single loci in other LGs were outliers on a regional scale (Table S2, Supporting information). Comparison with analysis of the historical Greenlandic samples suggested that this pattern was stable over time, although there were 30% fewer outliers among historical samples (Table S2, Supporting information).

Pairwise comparisons between the clusters showed that LG7 loci were only outliers in tests involving the Iceland inshore group (Fig. 4). The majority of global outliers in LG1 were outliers in all comparisons involving the “Iceland-inshore” and the “Nuuk” clusters, except in the comparison

of these two, indicating a common divergence from the other clusters at this genomic region (Fig. 4c). The smallest number of outliers was found in the “West”-“East” comparison, but the outliers here were in different LGs, thus likely representing independent instances of genomic divergence. Few significant outliers were detected within clusters, except from a few cases in both the “East” and “West” historical samples.

Temporal outlier detection

The temporal outlier analyses revealed between 3 and 9 outlier loci, mostly spread over multiple LGs, showing elevated levels of differentiation between time points within a location (Fig. 5; Table S2, Supporting information). Interestingly, there was no overlap between the loci that were temporal outliers in the different locations and only three loci were both spatial and temporal outliers. Uncertainty in the estimated parameter input values appeared to only have minor influence on the outlier detection. Assuming that the generation length was 7 years instead of 5 narrowed the confidence limits on simulated F_{temp} and produced a few more outliers. Using the lower 95% confidence limit rather than the point estimate for N_e generated slightly wider confidence intervals and consequently removed a few outliers. However, at least the top three temporal outliers for all locations were highly robust to variations in parameter inputs.

Environmental correlations

The BAYENV analysis identified between 1 and 29 loci that were highly correlated with the environmental variables (Table S2). All but two of the significantly correlated loci were also identified as spatial or temporal outliers. The high LD-group on LG1 that exhibited strong spatial outlier patterns correlated with a number of variables, including distance to shore, sea surface temperature range, and salinity. The spatial outlier loci on LG7 were correlated with longitude, which is expected given that these loci seemed divergent only between the Iceland inshore cluster and the rest. However, a number of additional loci distributed across LGs also correlated with longitude. Different sets of loci—some on LG1—correlated with maximum and mean bottom temperature, whereas a consistent set of 4 loci correlated with minimum and mean surface temperature. Three of these loci were involved in differentiation between the Iceland inshore and particularly the Nuuk samples (Table S2, Supporting information).

DISCUSSION

This study identified four genetically distinct groups inhabiting a relatively small geographical area at the Northern range margin of the widely distributed Atlantic cod. Genomic analysis of contemporary and historical samples revealed that the groups exhibited different spatial distributions with considerable overlap and mixing and that the genetic composition at some spawning grounds was stable over time, whereas complete population replacement was evident at others. Signatures of elevated differentiation in certain genomic regions are consistent with adaptive divergence between the groups and significantly increased temporal changes at a subset of loci indicate that adaptation is ongoing.

Population structure and reproductive isolation

Our results suggested a relatively high degree of reproductive isolation among the four identified groups, as the majority of individuals are assigned to a single cluster with very high certainty. While the posterior membership probabilities of the DAPC analysis are not strictly equivalent to individual admixture proportions as estimated through commonly applied Bayesian clustering methods (e.g. Pritchard et al. 2000; Corander et al. 2008), they do reflect the proximity of individuals to different clusters. Hence, individuals with relatively even membership probabilities to multiple clusters could either carry uninformative genotypes in relation to cluster separation or be admixed. In contrast, population samples that exhibited an intermediate average position between clusters but are made up of individuals with high membership probability to different clusters likely represent first-generation or non-interbreeding mixtures. Our finding that the latter scenario was much more common than the former (Fig. 3) does not appear to be an artifact of model over-fitting that would be able to distinguish any groupings with high power because re-analysis with randomized prior groupings resulted in maximum individual membership probabilities of only 0.3-0.5 for the vast majority of individuals (Fig. S5, Supporting information). Therefore, the data strongly suggest that spatial mixture among separate genetic clusters was common, but individual admixture much less so in this system.

A high degree of reproductive isolation could appear at odds with the relatively weak level of genetic structure observed when outlier loci were removed (Fig. S1, Supporting information). However, the differentiation between clusters was highly significant, and low levels of differentiation—a typical pattern for marine fish (Waples 1998; Hauser and Carvalho 2008)—does not necessarily reflect substantial ongoing gene flow. Our analysis suggested that the N_e was very large in all populations and previous ecological niche modeling coupled with genetic analysis indicates that the split between Greenlandic and Icelandic/European cod populations postdates the last glacial maximum (LGM; c. 21 KYA; (Bigg et al. 2008). Therefore, the low level of differentiation may be better explained by limited accumulation of drift due to recent divergence and large N_e 's.

The configuration of our inferred genetic clusters is consistent with previous hypotheses about population structure based on tagging data, abundance records, and egg distribution surveys, which also have indicated the presence of four components: an inshore west, offshore west, offshore east and inflow from Iceland (summarized by Buch et al. 1994; Storr-Paulsen et al. 2004). Among the inshore west coast samples, the genetic isolation of the Nuuk region also corroborates insights from egg surveys and historical records, which suggest that this is one of the most important inshore spawning areas (Storr-Paulsen et al. 2004). It is uncertain to what extent the portions of other samples that assigned to the Nuuk cluster represent related individuals from an inshore component distributed all along the coast or show similarity because of common adaptations to the inshore environment (see below).

Regardless of this uncertainty, this study provides important confirmation of the genetic basis of previously assumed population structure. Notably, the combination of extensive sampling at the spawning grounds and a large panel of SNP markers provided much greater power to resolve these patterns than previous genetic studies in the region have achieved (Arnason et al. 2000; O Leary et al. 2007; Pampoulie et al. 2011). The DAPC proved a powerful approach for detecting the weak, but geographically and biologically meaningful, signal of differentiation. The low differentiation made results from the more commonly applied Bayesian clustering algorithm STRUCTURE (Pritchard et al. 2000; Falush et al. 2003) less conclusive (not shown), and DAPC may in many cases be a suitable alternative (Jombart et al. 2010).

Temporal stability of population structure and distribution

In addition to characterizing the number of cod populations around Greenland, our spatiotemporal analysis provided important insights into how the distribution of the different components has changed over time. Perhaps most interesting was the demonstration of genetic continuity on the west coast banks. After the stock collapse in the late 1960s, cod were considered virtually extinct from the offshore regions and it was hypothesized that influx from Iceland would be the only viable source of replenishment (Rätz et al. 1999; Stein 2007). Here we show that recently collected cod from these offshore areas represent an almost identical mixture of fish with western and eastern Greenlandic heritage as was sampled there during the period of maximum abundance. Although this population component probably now is recovering from a severe reduction in population size, our temporal analysis indicated that the N_e has remained high and thus that the population is unlikely to have suffered alarming loss of genetic diversity—a pattern also observed in other large cod populations that have undergone substantial population collapses (Ruzzante et al. 2001; Poulsen et al. 2006; Therkildsen et al. 2010b). Since the distribution of the western Greenland cluster extends to coastal areas where a lower level abundance was maintained (Buch et al. 1994; Storr-Paulsen et al. 2004), it cannot be ruled out that the offshore area was re-colonized by a population component that had resided inshore. However, it appears virtually impossible that the offshore resurgence has resulted exclusively from Icelandic influx.

Interestingly, all the historical coastal samples outside the “Nuuk” area show remarkable similarity (also with one of the offshore areas (LHB, see Figs. 1, 2 and 3), but in contemporary time this “pure” west coast cluster is only represented at the northernmost location. At SIS, recent samples were more influenced by the “Nuuk” cluster although they still contained a considerable number of individuals assigning to the “West” cluster. Interestingly, the 5-year temporal replicates at both historical and contemporary time in this location indicate that the proportional representation of the different clusters maintained short-term stability.

In the southern coastal locations (PAA and QAQ), that historically showed genetic similarity to the other coastal locations, the “West” cluster became entirely replaced by fish from the “Iceland-inshore” and “East” cluster. This shifting pattern is consistent with observations of periodic larval drift across the Denmark Strait (Wieland and Hovgård 2002), but the complete replacement is perhaps surprising. Also, tagging studies have suggested that Icelandic fish migrate back to Iceland to spawn and not contribute to recruitment in Greenland. However, the fish analyzed here were in spawning condition and thus a large proportion could reproduce locally with uncertain consequences for future separation and distribution of the genetic groups. Clearly, the data demonstrate highly dynamic patterns with large temporal shifts in the distribution and overlap among clusters. Ongoing investigations including samples collected at a finer spatial resolution within key locations may reveal what factors drive these changes.

Adaptive divergence and evolutionary potential

The consistent results from 1) the two independent outlier tests, 2) the loading plots from the DAPC, and 3) the correlations with seascape variables indicate strong effects of divergent selection in this system. In some cases the signatures of selection were found *within*, but primarily they were evident *between* the four clusters. This is consistent with cluster-specific adaptations to local conditions. The observation that contrasting genomic regions showed elevated divergence across different cluster pairs in turn indicates that different genes may underlay the adaptive response to different environments.

The vast majority of outlier loci were located within three genomic regions that span up to >20cM on the linkage map and exhibit strong LD within all samples. Consistent with “islands of genomic

divergence” against a background of lower levels of differentiation (Turner et al. 2005; Nosil et al. 2009) , the tight clustering of outliers in the genome also supports that our findings reflect real patterns of adaptive genetic divergence, not just spurious statistical outliers. Further, the identified outlier regions—and in many cases the same particular SNPs—have also been shown to exhibit highly elevated divergence in other parts of the species range over both small and large spatial scales (Nielsen et al. 2009; Bradbury et al. 2010; Poulsen et al. 2011), confirming their affiliation with local adaptation.

Identifying the specific targets of selection in these regions that contain 100s of genes, and elucidating the mechanisms behind their fitness effects, will require detailed experiments (Stinchcombe and Hoekstra 2007; Barrett and Hoekstra 2011). However, our analysis here suggested that the allele frequencies of several loci correlate with spatial variation for a number of environmental variables. The highest number of correlations was found for longitude. Longitudinal patterns were strongly driven by the difference between Iceland and Greenland and one of the major differences between these two areas is the overall temperature regime. The role of temperature in shaping allele frequencies in these loci is further supported by a previous study that also reported temperature-associated clines on both sides of the Atlantic for many of the same loci (Bradbury et al. 2010). The direct temperature variables included in the analysis correlated with fewer SNPs than did longitude (though some very strongly). However, as inherent to all correlation analyses, it is difficult to know exactly whether a summarized variable captures the biologically relevant aspect of environmental variation.

A perhaps more robust proxy, distance to shore, showed a very strong correlation with the outlier loci in LG1, including the well-studied *Pan-I* polymorphism, for which inshore-offshore divergence has also been demonstrated in Iceland and Norway (Fevolden and Pogson 1997; Pampoulie et al. 2006; Wennevik et al. 2008). Here, this genomic region shows parallel allele frequency differences between inshore and offshore samples in both Iceland and Greenland and the DAPC discrimination between these groups of samples were almost exclusively driven by loci from this group (Fig. S4b, Supporting information). The pattern is so pronounced that with the full SNP panel, the K=3 clustering solution grouped the “Nuuk” and “Iceland-inshore” samples together. With strong outliers and high-LD loci removed, the “Nuuk” samples show approximately equal affiliation with Greenlandic and the Icelandic clusters (Fig. S3b, Supporting information): However, with the conservative criteria for detecting outliers applied here, a number of residual signatures of weaker selection may remain in this presumably “neutral” data set, leaving the demographic history of the Nuuk cluster somewhat confounded.

Although the specific drivers and mechanisms are only partly resolved, our results clearly indicate that the four clusters exhibit different adaptations and therefore may respond differently to climate change. If the Greenlandic clusters only split from Iceland after the LGM, the observed signatures of selection have all arisen recently on an evolutionary time scale, suggesting a high evolutionary potential within the species. The observation of a higher number of outliers in contemporary compared to historical samples within Greenland could also indicate ongoing adaptation over the study period, although this pattern may also be partly caused by issues of statistical power related to the not completely identical sampling schemes in time. Further evidence for ongoing spatial adaptation was found in the increased temporal differentiation at particular loci in the locations where the presumably neutral genetic composition had been stable. Since the set of temporal outliers was generally non-overlapping with spatial outliers (indicating lack of spatial variation in allele frequencies at these loci), migration is unlikely to have caused the differentiation that exceeds expectations based on drift and sampling error. Therefore, ongoing selection seems the most parsimonious explanation, indicating signs of adaptive changes over decadal time scales.

Conclusion

Overall, our results illustrate the complex and dynamic interactions of four genetically distinct groups of cod inhabiting the northern range margin of the species. The different groups of cod already exhibit signs of adaptive divergence and show potential for rapid response to ongoing changes in selection pressures. Temporal variations in the genetic composition at different locations suggest that the groups respond differently to environmental variation, although the continued presence of all components despite major demographic changes indicates considerable resilience. Accordingly, population diversity and evolutionary potential should clearly be taken into account in attempts to model or predict species-level shifts to more northern habitats. The observed population variability can generate complementary dynamics among population components, so-called portfolio effects (Schindler et al. 2010), which may prove critical for ensuring the persistence and stability of both the species and future fisheries yields. Conservation and resource management efforts should thus carefully consider and aim to protect the full biocomplexity of the system, and spatiotemporal population genomics studies provide a powerful tool for informing such undertakings.

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LITERATURE CITED

- Arnason, E., P. H. Petersen, K. Kristinsson, H. Sigurgislason, and S. Pálsson. 2000. Mitochondrial cytochrome b DNA sequence variation of Atlantic cod from Iceland and Greenland. *Journal of Fish Biology* **56**:409–430.
- Barrett, R. D. H., and H. E. Hoekstra. 2011. Molecular spandrels: tests of adaptation at the genetic level. *Nature Reviews Genetics* **12**:767–780.
- Beaumont, M. A., and D. J. Balding. 2004. Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology* **13**:969–980.
- Beaumont, M. A., and R. A. Nichols. 1996. Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London Series B-Biological Sciences* **263**:1619–1626.
- Bigg, G. R., C. W. Cunningham, G. Ottersen, G. H. Pogson, M. R. Wadley, and P. Williamson. 2008. Ice-age survival of Atlantic cod: agreement between palaeoecology models and genetics. *Proceedings of the Royal Society of London Series B-Biological Sciences* **275**:163–173.
- Borza, T., B. Higgins, G. Simpson, and S. Bowman. 2010. Integrating the markers Pan I and haemoglobin with the genetic linkage map of Atlantic cod (*Gadus morhua*). *BMC Research Notes* **3**:261.
- Bowman, S., S. Hubert, B. Higgins, C. Stone, J. Kimball, T. Borza, J. T. Bussey, G. Simpson, et al. 2011. An integrated approach to gene discovery and marker development in Atlantic cod (*Gadus morhua*). *Marine Biotechnology* **13**:242–255.
- Bradbury, I. R., S. Hubert, B. Higgins, T. Borza, S. Bowman, I. G. Paterson, P. V. R. Snelgrove, C. J. Morris, et al. 2010. Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. *Proceedings of the Royal Society of London Series B-Biological Sciences* **277**:3725–3734.
- Buch, E., S. A. Horsted, and H. Hovgård. 1994. Fluctuations in the occurrence of cod in Greenland waters and their possible causes. *ICES Mar. Sci. Symp.* **198**:158–174.

- Burrows, M. T., D. S. Schoeman, L. B. Buckley, P. Moore, E. S. Poloczanska, K. M. Brander, C. Brown, J. F. Bruno, et al. 2011. The pace of shifting climate in marine and terrestrial ecosystems. *Science* **334**:652–655.
- Chen, I. C., J. K. Hill, R. Ohlemuller, D. B. Roy, and C. D. Thomas. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science* **333**:1024–1026.
- Corander, J., P. Marttinen, J. Sirén, and J. Tang. 2008. Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinformatics* **9**:539.
- Drinkwater, K. 2009. Comparison of the response of Atlantic cod (*Gadus morhua*) in the high-latitude regions of the North Atlantic during the warm periods of the 1920s–1960s and the 1990s–2000s. *Deep-Sea Research Part II* **56**:2087–2096.
- Drinkwater, K. F. 2005. The response of Atlantic cod (*Gadus morhua*) to future climate change. *ICES Journal of Marine Science* **62**:1327–1337.
- Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**:564–567.
- Excoffier, L., T. Hofer, and M. Foll. 2009. Detecting loci under selection in a hierarchically structured population. *Heredity* **103**:285–298.
- Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**:1567–1587.
- Fevolden, S. E., and G. H. Pogson. 1997. Genetic divergence at the synaptophysin (Syp I) locus among Norwegian coastal and north-east Arctic populations of Atlantic cod. *Journal of Fish Biology* **51**:895–908.
- Foll, M., and O. Gaggiotti. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics* **180**:977–993.
- Gienapp, P., C. Teplitsky, J. S. Alho, J. A. Mills, and J. Merilä. 2008. Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology* **17**:167–178.
- Guillot, G., F. Mortier, and A. Estoup. 2005. GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes* **5**:712–715.
- Hansen, P. 1949. Studies on the biology of the cod in Greenland waters. *Rapp. P.-v. Réun. Cons. Int. Explor. Mer* **123**:1–77.
- Hauser, L., and G. R. Carvalho. 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. *Fish and Fisheries* **9**:333–362.
- Hemmer-Hansen, J., E. E. Nielsen, D. Meldrup, and C. Mittelholzer. 2011. Identification of single nucleotide polymorphisms in candidate genes for growth and reproduction in a nonmodel organism; the Atlantic cod, *Gadus morhua*. *Molecular Ecology Resources* **11**:71–80.
- Hilborn, R., T. P. Quinn, D. E. Schindler, and D. E. Rogers. 2003. Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Sciences of the United States of America* **100**:6564–6568.
- Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. *Nature* **470**:479–485.
- Horsted, S. A. 2000. A review of the cod fisheries at Greenland, 1910–1995. *Journal of Northwest Atlantic Fishery Science* **28**.
- Hubert, S., B. Higgins, T. Borza, and S. Bowman. 2010. Development of a SNP resource and a genetic linkage map for Atlantic cod (*Gadus morhua*). *BMC Genomics* **11**:191.
- ICES. 2011. Report of the North Western Working Group (NWWG), 26 April - 3 May 2011, ICES Headquarters, Copenhagen.
- Jeffreys, H. 1939. *Theory of Probability*. Clarendon Press, Oxford.
- Jombart, T. 2008. ADEGENET: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**:1403–1405.
- Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11**:94.
- Kelly, M. W., E. Sanford, and R. K. Grosberg. 2011. Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proceedings of the Royal Society of London Series B-Biological Sciences* **279**:349–356.
- Moen, T., B. Hayes, F. Nilsen, M. Delghandi, K. T. Fjalestad, S.-E. Fevolden, P. R. Berg, and S. Lien. 2008. Identification and characterisation of novel SNP markers in Atlantic cod: Evidence for directional selection. *BMC Genetics* **9**:18.
- Nielsen, E. E., A. Cariani, E. M. Aoidh, G. E. MAES, I. Milano, R. Ogden, M. I. Taylor, J. Hemmer-Hansen, et al. 2012. Gene-associated markers provide tools for tackling illegal fishing and false eco-certification. *Nature Communications* **3**:851.
- Nielsen, E. E., J. Hemmer-Hansen, N. A. Poulsen, V. Loeschcke, T. Moen, T. Johansen, C. Mittelholzer, G.-L. Taranger, et al. 2009. Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). *BMC Evolutionary Biology* **9**:276.
- Nosil, P., D. J. Funk, and D. Ortiz-Barrientos. 2009. Divergent selection and heterogeneous genomic divergence. *Molecular Ecology* **18**:375–402.
- O Leary, D. B., J. Coughlan, E. Dillane, T. V. McCarthy, and T. F. Cross. 2007. Microsatellite variation in cod *Gadus morhua* throughout its geographic range. *Journal of Fish Biology* **70**:310–335.
- Pampoulie, C., A. K. Danielsdottir, M. Storr-Paulsen, H. Hovgård, E. Hjörleifsson, and B. Æ. Steinarsson. 2011. Neutral and nonneutral genetic markers revealed the presence of inshore and offshore stock components of Atlantic cod in Greenland waters. *Transactions of the American Fisheries Society* **140**:307–319.
- Pampoulie, C., D. E. Ruzzante, V. Chosson, T. D. Jorundsdottir, L. Taylor, V. Thorsteinsson, A. K. Danielsdottir, and G.

- Marteinsdottir. 2006. The genetic structure of Atlantic cod (*Gadus morhua*) around Iceland: insight from microsatellites, the *Pan I* locus, and tagging experiments. *Canadian Journal of Fisheries and Aquatic Sciences* **63**:2660–2674.
- Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**:37–42.
- Poulsen, N. A., E. E. Nielsen, M. H. Schierup, V. Loeschcke, and P. Grønkjær. 2006. Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). *Molecular Ecology* **15**:321–331.
- Poulsen, N., J. Hemmer-Hansen, and V. Loeschcke. 2011. Microgeographical population structure and adaptation in Atlantic cod *Gadus morhua*. *Marine Ecology Progress Series* **436**:231–243.
- Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**:945–959.
- Rätz, H., and J. Lloret. 2005. Long-term variability of growth and recruitment of cod (*Gadus morhua*) off Greenland. *ICES Journal of Marine Science* **62**:1310–1321.
- Rätz, H., M. Stein, and J. Lloret. 1999. Variation in growth and recruitment of Atlantic cod (*Gadus morhua*) off Greenland during the second half of the twentieth century. *Journal of Northwest Atlantic Fishery Science* **25**:161–170.
- Ruzzante, D. E., C. T. Taggart, R. W. Doyle, and D. Cook. 2001. Stability in the historical pattern of genetic structure of Newfoundland cod (*Gadus morhua*) despite the catastrophic decline in population size from 1964 to 1994. *Conservation Genetics* **2**:257–269.
- Ryman, N. 2006. CHIFISH: a computer program testing for genetic heterogeneity at multiple loci using chi-square and Fisher's exact test. *Molecular Ecology Notes* **6**:285–287.
- Schindler, D. E., R. Hilborn, B. Chasco, C. P. Boatright, T. P. Quinn, L. A. Rogers, and M. S. Webster. 2010. Population diversity and the portfolio effect in an exploited species. *Nature* **465**:609–612.
- Star, B., A. J. Nederbragt, S. Jentoft, U. Grimholt, M. Malmstrøm, T. F. Gregers, T. B. Rounge, J. Paulsen, et al. 2011. The genome sequence of Atlantic cod reveals a unique immune system. *Nature* **477**:207–210.
- Stein, M. 2007. Warming periods off Greenland during 1800–2005: their potential influence on the abundance of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) in Greenlandic waters. *Journal of Northwest Atlantic Fishery Science* **39**:1–20.
- Stinchcombe, J. R., and H. E. Hoekstra. 2007. Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. *Heredity* **100**:158–170.
- Storey, J. D., and R. Tibshirani. 2003. Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences of the United States of America* **100**:9440–9445.
- Storr-Paulsen, M., K. Wieland, H. Hovgård, and H. Rätz. 2004. Stock structure of Atlantic cod (*Gadus morhua*) in West Greenland waters: implications of transport and migration. *ICES Journal of Marine Science* **61**:972–982.
- Therkildsen, N. O., E. E. Nielsen, K. Hüsey, D. Meldrup, and A. J. Geffen. 2010a. Does DNA extraction affect the physical and chemical composition of historical cod (*Gadus morhua*) otoliths? *ICES Journal of Marine Science* **67**:1251–1259.
- Therkildsen, N. O., E. E. Nielsen, D. P. Swain, and J. S. Pedersen. 2010b. Large effective population size and temporal genetic stability in Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences* **67**:1585–1595.
- Turner, T. L., M. W. Hahn, and S. V. Nuzhdin. 2005. Genomic islands of speciation in *Anopheles gambiae*. *PLoS Biology* **3**:e285.
- Waples, R. S. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity* **89**:438–450.
- Warnes, G. 2003. The genetics package. *R News* **3**:9–13.
- Wennevik, V., K. E. Jorstad, G. Dahle, and S.-E. Fevolden. 2008. Mixed stock analysis and the power of different classes of molecular markers in discriminating coastal and oceanic Atlantic cod (*Gadus morhua* L.) on the Lofoten spawning grounds, Northern Norway. *Hydrobiologia* **606**:7–25.
- Wieland, K., and H. Hovgård. 2002. Distribution and drift of Atlantic cod (*Gadus morhua*) eggs and larvae in Greenland offshore waters. *Journal of Northwest Atlantic Fishery Science* **30**:61–76.

FIGURE LEGENDS

Fig. 1. Approximate sampling locations in Greenland and Iceland. Dots shifted left represent historical samples while dots shifted right represent contemporary samples (see Table 1 for years). The colors of dots represent the sample mean coordinate on the two first DAPC discriminant functions recoded as signal intensities of red and green, respectively (see text and Fig. 2).

Fig. 2. Scatterplots of the mean sample coordinates on the first and second (a) and the first and third (b) discriminant functions (DF) from the DAPC based on the four inferred clusters. Contemporary sample names are plotted in black and historical sample names in grey. The colored bars along the first and second DF illustrate the color recoding in intensity of red and green shown in Fig. 1 (see text).

Fig. 3. Plot of the posterior membership probabilities of each individual to the Iceland inshore (yellow), East (red), West (green), and Nuuk (brown) clusters, respectively. Each vertical line represents an individual and is divided into color segments proportional to its posterior membership probability to each of the geographic clusters derived from the DAPC including only the “pure” samples (see text). The order of individuals within samples is random, but samples are ordered according to hydrographic distance from the easternmost sample.

Fig. 4. Matrix of results from the BayeScan spatial outlier tests in pairwise comparisons of the clusters. Column and row headers indicate the cluster pair compared in each cell. Each circle represents a locus and loci to the right of the vertical bars (representing $q = 0.05$) are considered significant outliers. Loci that were also outliers in the Arlequin analysis are marked by filled circles colored blue for loci in LG1, red for loci in LG7 and black for loci in all other LG's.

Fig. 5. Examples of temporal outlier detection results in DAB (a) and KAP (b). Each dot represents a locus, illustrating its temporal differentiation (F_{temp} , y-axis) against its H_e (x-axis). The lines represent the 95% (grey) and the 99% (black) confidence envelopes of the simulated neutral distribution.

AUTHOR CONTRIBUTIONS BOX

NOT and EEN designed the research with input from MSW, RBH and AR. RBH and AR contributed samples and ecological data. MSW contributed environmental data. NOT performed the molecular analyses and analyzed the data with input from EEN and JHH. NOT wrote the paper with input from all authors.

Table 1. Summary of sample information including the sampling year, the cluster to which most individuals assigned and the mean posterior membership probability to that cluster (see text), the number of individuals (n), and diversity statistics. Samples are ordered by hydrographic distance from the easternmost sample

Country	Location	Year	Abbreviation	Region	Cluster	Mrship Probability	n	Variable Loc	Missing data (%)	H _{obs}	H _e
Iceland	Northeast coastal	2002	INC02	Coastal	Iceland-inshore	0.69	39	841	0.7	0.27	0.27
	Southwest coastal	2002	ISC02	Coastal	Iceland-inshore	0.90	38	845	1.0	0.27	0.27
	Southwest offshore	2002	ISO02	Offshore	East	0.83	39	851	0.8	0.26	0.26
Greenland	Tasiilaq	2010	TAS10	Coastal	East	0.69	29	807	13.7	0.28	0.25
	Offshore East	2010	OEA10	Offshore	East	0.96	29	810	6.0	0.27	0.25
	Offshore South	2010	OSO10	Offshore	East	0.96	29	807	11.0	0.28	0.26
	Danas Banke	1934	DAB34	Offshore	East	0.52	31	926	3.2	0.29	0.29
		2008	DAB08	Offshore	East	0.67	21	857	3.5	0.26	0.27
	Fyllas Banke	1954	FYB54	Offshore	East	0.50	30	891	3.6	0.28	0.28
	Qaqortoq	1947	QAQ47	Coastal	West	0.82	28	914	4.6	0.31	0.32
		2008	QAQ08	Coastal	East	0.39	27	854	3.5	0.26	0.27
	Paamiut	1947	PAA47	Coastal	West	0.87	31	917	4.9	0.33	0.31
		2008	PAA08	Coastal	East	0.49	29	850	3.0	0.27	0.27
	Ameralik	2008	AME08	Fjord	Nuuk	0.79	30	891	3.8	0.29	0.30
	Qorqut	2008	QOR08	Fjord	Nuuk	0.76	30	901	3.7	0.30	0.29
	Kapisillit	1943	KAP43	Fjord	Nuuk	0.77	30	894	4.2	0.30	0.29
		2008	KAP08	Fjord	Nuuk	0.75	30	902	1.5	0.30	0.30
	Offshore West	2010	OWE10*	Offshore	West	0.56	39	910	2.1	0.29	0.29
	Lille Hellefiskebanke	1957	LHB57	Offshore	West	0.81	31	912	5.3	0.30	0.31
	Store Hellefiskebanke	1950	SHB50	Offshore	West	0.37	31	909	1.7	0.29	0.30
	Sisimiut	1932	SIS32	Coastal	West	0.99	20	876	7.8	0.33	0.31
		1937	SIS37	Coastal	West	0.96	31	891	4.8	0.29	0.30
		2005	SIS05**	Coastal	West	0.42	34	919	1.7	0.31	0.31
	Ilulissat	2010	SIS10	Coastal	West	0.50	26	892	13.7	0.34	0.30
		1953	ILL53	Coastal	West	0.80	30	898	3.8	0.31	0.31
		2010	ILL10	Coastal	West	0.65	30	902	3.7	0.31	0.30
	Uummannaq	1945	UMM45	Coastal	West	0.73	30	898	3.7	0.31	0.31
		2010	UMM10	Coastal	West	0.95	25	891	11.1	0.35	0.31
Mean						0.72	30	881	4.7	0.29	0.29

*Due to the absence of contemporary spawning aggregations offshore, the individuals in this sample were collected over the entire west coast area. The mean position is plotted in Fig. 1

** This is the only sample collected outside the spawning season

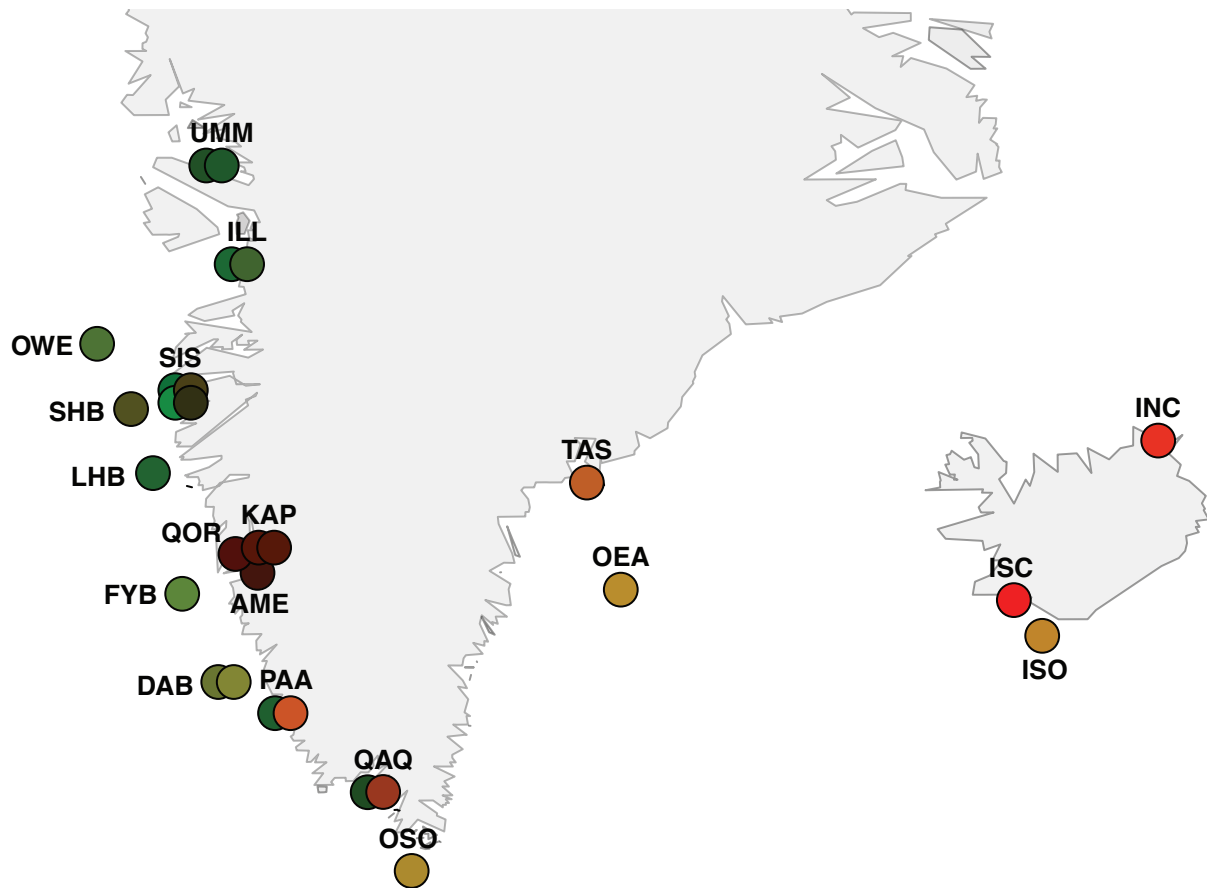


Fig. 1

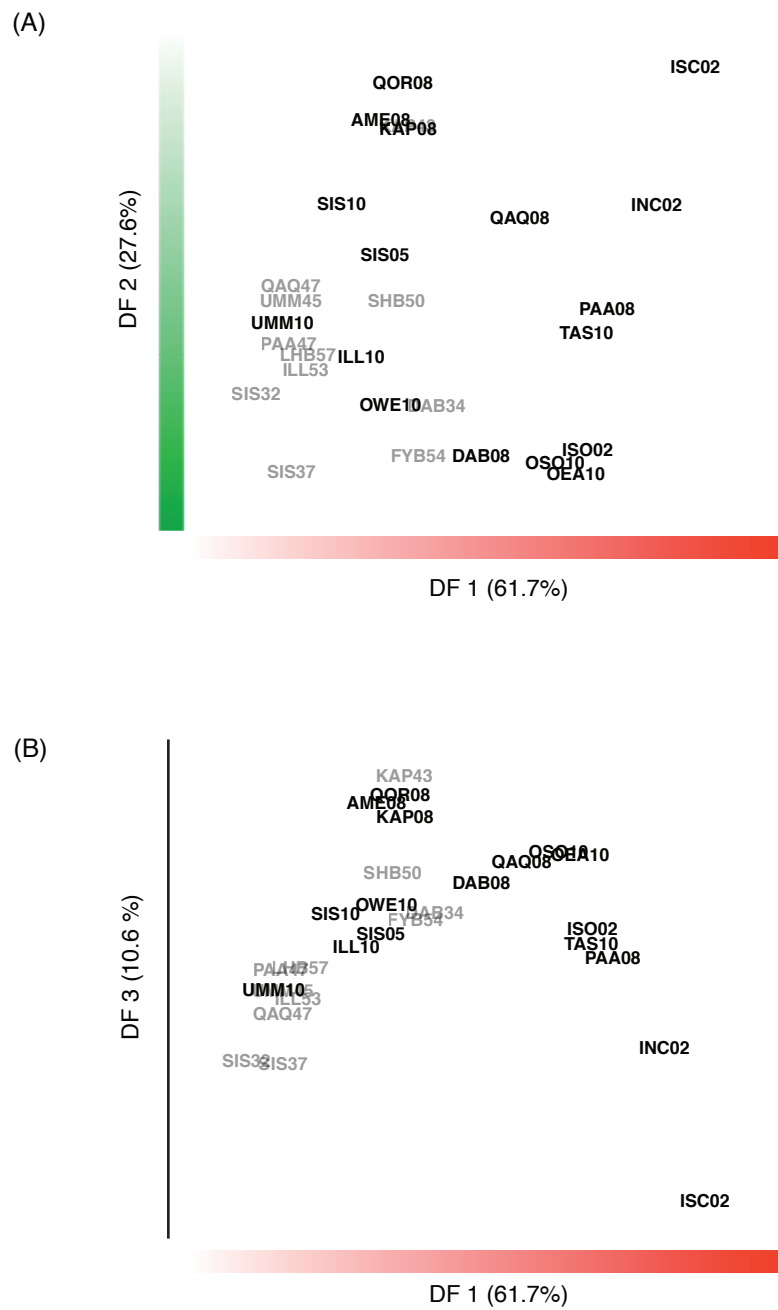


Fig. 2

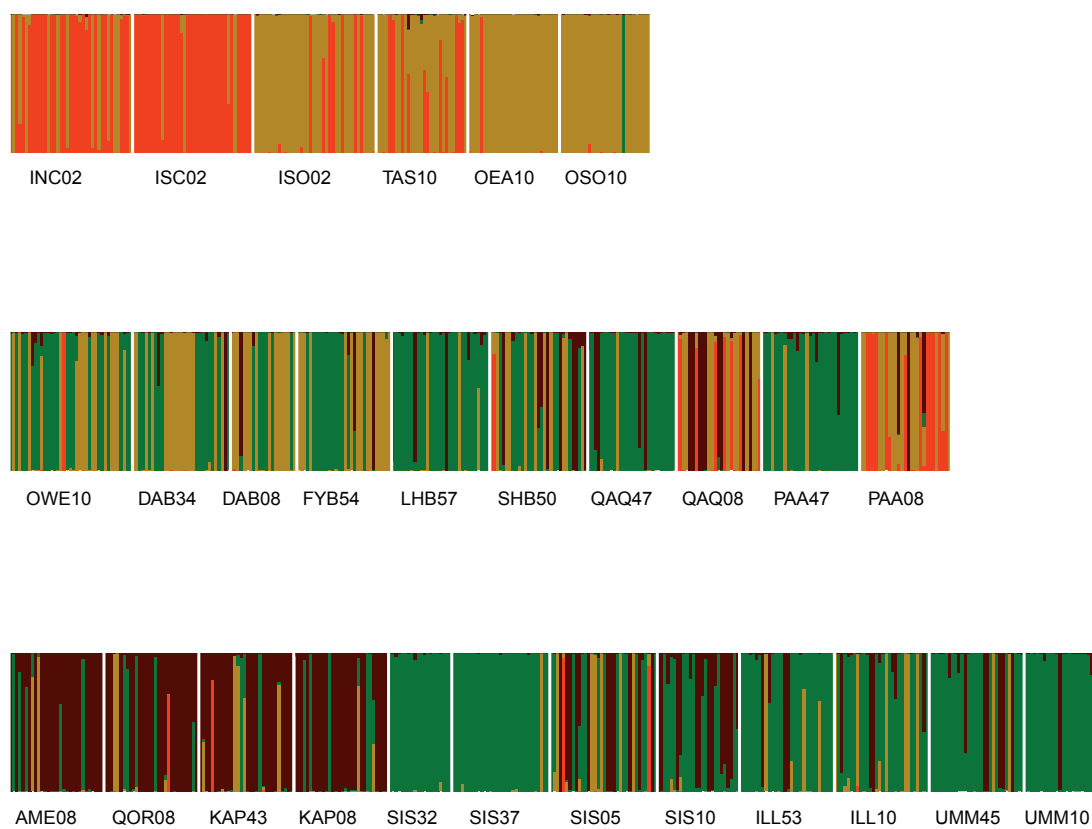


Fig. 3

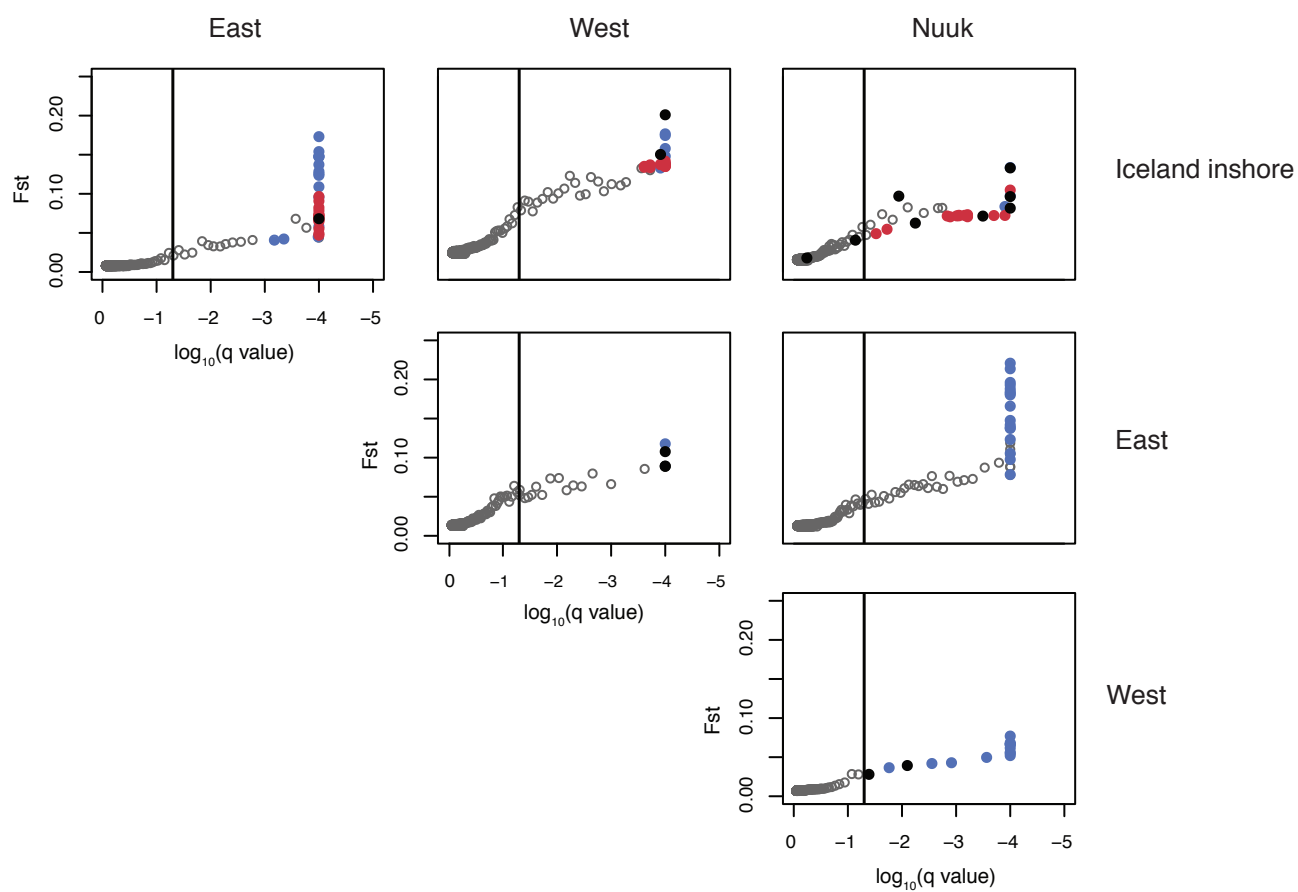


Fig. 4

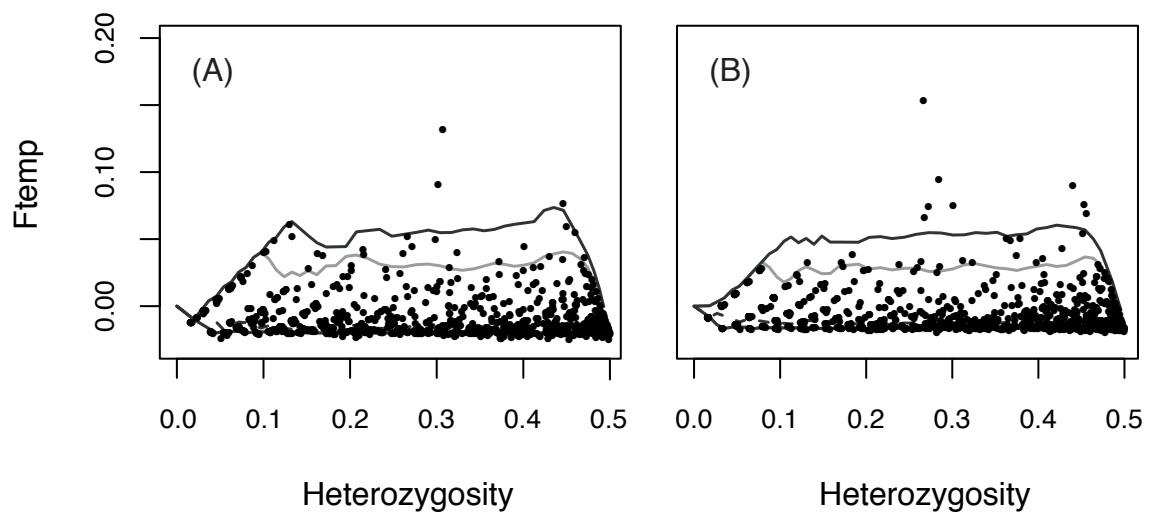


Fig. 5

Supporting information for the article

Spatiotemporal SNP analysis reveals pronounced biocomplexity at the northern range margin of Atlantic cod *Gadus morhua*

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Supplementary Note: Temporal outlier detection

Approach

To identify loci that showed elevated levels of differentiation among samples collected over time within single populations, we used a modified version of the *fdist* method (Beaumont and Nichols 1996) that is commonly applied for this purpose in spatial comparisons of samples collected from different populations at a single time point. Based on the premise that selection should affect only certain parts of the genome whereas neutral evolutionary forces should cause genome-wide effects, the method compares the observed locus-specific F_{ST} values as a function of heterozygosity (H_s) to a null distribution generated through simulations. Any loci that show divergent patterns of differentiation compared to this neutral expectation are then considered a candidate for being affected by selection.

Here, we adapted the method to fit our scenario by generating the expected neutral distribution through simulation of drift within a single population rather than as drift-migration equilibrium between multiple demes, as is implemented in the original formulation. Migration can have contrasting effects on allele frequencies within a population over short time scales depending on the level of differentiation between the source and the recipient populations (Wang and Whitlock 2003; Fraser et al. 2007) and this can be complex to generalize. Consequently, our null model included only the effects of drift and sampling within an isolated population. Assuming that the time scale considered in this study (up to 15 generations) is sufficiently short to ignore the effects of mutations, any departure from the null model expectations is then likely caused either by selection or gene flow.

Model and parameter inputs

Our simulations were based on single bi-allelic loci at initial frequency f_0 in a Wright-Fisher population of constant size, N_e , that reproduced over t generations. At generation zero and generation t , a sample of size n individuals was collected. We ran the analysis separately for each of the locations that showed temporal stability in cluster assignment, each time parameterizing the model to most closely match the studied scenario.

The initial allele frequency f_0 at each simulated locus was a random number between 0 and 1, but to generate a roughly uniform distribution of H_s values among the simulated loci, we enriched for low starting frequencies. The input parameters N_e , t and n were adjusted for each location based on estimates from the data.

The sample size n was the harmonic mean of sample sizes for the location. To convert the number of years to the number of generations between samples (t), we estimated the generation length as the mean age of spawners weighted by age-specific fecundity following Miller and Kapuscinski (1997). These calculations were based on abundance-at-age and weight-at-age data from annual surveys 1982-2010 (ICES 2011), coupled with maturity- and fecundity-at-weight data (Hedeholm unpublished data). The spatial resolution of the data only allowed for a single inshore and a single offshore estimate. In both cases, the generation length was approximated to be around 5 years, implying that the sampling interval for temporal replicates spanned 11-15 generations.

We estimated the N_e for each location based on the temporal variance in allele frequencies between sampling points using the estimator of Waples (1989), as implemented in the software NeEstimator (Peel et al. 2004). Because N_e estimates from genetic data can be biased downward

with inclusion of loci under directional selection (Leberg 2005; Wang 2005), we conducted the analysis iteratively, first basing simulations on the initial N_e estimates, then re-estimating the N_e without the temporal outlier loci detected in this first run, and basing final simulations on these adjusted N_e estimates. This estimation procedure suggested that the N_e was very large in all locations with lower 95% confidence limits on estimates consistently ≥ 450 . For the locations with point estimates of infinity (indicating a size larger than the method could quantify), we used an N_e of 10000 as input for the F_{temp} simulations.

Outlier identification

We quantified the temporal variance in allele frequencies F_{temp} between all samples from a population in both the observed and simulated data with Wright's F (Wright 1951), correcting for sampling effects following Waples (1998):

$$F_{temp} = \frac{\text{var}(p)}{\bar{p}(1 - \bar{p})} - \frac{1}{2n}$$

The correction for sampling effects was important because missing data made the actual sample size vary between loci in the observed data. Following Beaumont and Nichols (1996), we plotted F_{temp} as a function of the H_s for each locus. We simulated 100,000 independent loci and for each computed paired values of F_{temp} and H_s . As in the *fdist* method, the paired values were rank-ordered by H_s and grouped into overlapping bins of 4,000 points centered on every 2,000th point. For each bin, we computed the quantiles of the distribution of F_{temp} values that would define the confidence envelopes in which 95% and 99%, respectively, of the data points were expected to lie if behaving according to the model. To assess the statistical significance of departures from the neutral expectation, empirical p-values were computed for each locus as the proportion of simulated data points within its bin that showed higher F_{temp} than the observed value. To control the false discovery rate to $<5\%$, we also computed q-values for all loci using the R-package *qvalue* (Storey and Tibshirani 2003). All simulations and computations were completed with custom R-scripts (available upon request).

Literature cited

- Beaumont, M. A., and R. A. Nichols. 1996. Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London Series B-Biological Sciences* **263**:1619–1626.
- Fraser, D. J., M. M. Hansen, S. Østergaard, N. Tessier, M. Legault, and L. Bernatchez. 2007. Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. *Molecular Ecology* **16**:3866–3889.
- ICES. 2011. Report of the North Western Working Group (NWWG), 26 April - 3 May 2011, ICES Headquarters, Copenhagen.
- Leberg, P. 2005. Genetic approaches for estimating the effective size of populations. *Journal of Wildlife Management* **69**:1385–1399.
- Miller, L. M., and A. R. Kapuscinski. 1997. Historical analysis of genetic variation reveals low effective population size in a northern pike (*Esox lucius*) population. *Genetics* **147**:1249–1258.
- Peel, D., J. R. Ovenden, and S. L. Peel. 2004. NeEstimator: software for estimating effective population size, Version 1.3.
- Storey, J. D., and R. Tibshirani. 2003. Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences of the United States of America* **100**:9440–9445.

- Wang, J. 2005. Estimation of effective population sizes from data on genetic markers. *Philosophical Transactions of the Royal Society B: Biological Sciences* **360**:1395–1409.
- Wang, J. L., and M. C. Whitlock. 2003. Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* **163**:429–446.
- Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* **121**:379–391.
- Waples, R. S. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity* **89**:438–450.
- Wright, S. 1951. The genetical structure of populations. *Annals of Eugenics* **15**:323–353.

Table S1. List of variables initially considered for environmental correlation analysis. Variables that were retained for BAYENV analysis are marked by "x"

Variables	Used with BAYENV
Latitude	x
Longitude	x
Region (fjord, coastal, offshore)	
Distance to nearest shoreline	x
Distance to Iceland	
Maximum bottom temperature during spawning months	x
Mean bottom temperature during spawning months	x
Minimum bottom temperature during spawning months	
Range in bottom temperature during spawning months	x
Maximum annual bottom temperature	
Mean annual bottom temperature	
Minimum annual bottom temperature	
Maximum sea surface temperature during spawning months	
Mean sea surface temperature during spawning months	x
Minimum sea surface temperature during spawning months	x
Range in sea surface temperature during spawning months	x
Maximum annual sea surface temperature	
Mean annual sea surface temperature	
Minimum annual sea surface temperature	
Range in annual sea surface temperature	
Mean bottom salinity during spawning months	
Mean annual bottom salinity	
Mean surface salinity during spawning months	
Mean annual surface salinity	x

Table S2. Overview of outlier loci identified in the different neutrality tests. For the spatial outliers F_{ST} significant ($q < 0.05$) outliers that were identified both by BayeScan and Arlequin, and "+" indicates significant ($q < 0.05$) F_{CT} outliers identified under the hierarchical model. For temporal outliers "x" indicates loci that fell above the 99% confidence envelope of the expected neutral distribution. For the BayEnv results, the table indicates significant correlation ($\log_{10}(BF) > 1.5$) with environmental variables in tests with all samples ("x"), with only contemporary samples ("x+"), and with only historical samples ("x**")

Spatial outliers ¹										Temporal outliers										Correlations with environment ²											
Regional				Pairwise cluster comparisons								Within cluster																			
Name	LG	Pos	LD	All	Ice	GC	GH	I-E	I-W	I-N	W-E	N-E	N-W	I	E	W	N	DAB	FYB	ILL	KAP	UMM	DC	Lat	Long	MaB	MeB	MeS	MIS	RST	Sal
cgpGmo-S512	1	8.1																													
cgpGmo-S1788	1	12.8		x+															x												
cgpGmo-S968	1	14.6 *																													
cgpGmo-S985	1	15.6 *		x+																											
cgpGmo-S1703	1	16.6 *		x+																											
cgpGmo-S1196b	1	16.9 *		+																											
cgpGmo-S875b	1	18.1 *		x+																											
cgpGmo-S975b	1	18.1 *		x+																											
cgpGmo-S1268	1	19.3 *		x																											
cgpGmo-S1365b	1	19.7 *		+																											
cgpGmo-S1853	1	19.7 *		x+																											
cgpGmo-S1806	1	20.6 *		x+																											
Pan1	1	25.1 *		x+																											
cgpGmo-S83	1	26.6 *																													
cgpGmo-S852	1	26.8 *		x+																											
cgpGmo-S523	1	30.4 *		x																											
cgpGmo-S254	1	31.8 *																													
cgpGmo-S2082	1	33.1 *		x+																											
cgpGmo-S603	1	34.1 *		x+																											
cgpGmo-S360	1	35.8 *		x+																											
cgpGmo-S1845	1	37.2 *		x+																											
cgpGmo-S292b	1	38.6 *		x+																											
Gm394_0364	1			x+																											
cgpGmo-S1166	1			x+																											
cgpGmo-S1874	1			x+																											
cgpGmo-S1955	1			x+																											
cgpGmo-S2095	1			x+																											
Rhod_1_1	1			x+																											
cgpGmo-S754	2	1.0																													
cgpGmo-S155	2	17.4																													
cgpGmo-S728	2	26.3																													
cgpGmo-S1284	2	43.8																													
cgpGmo-S1026	2	49.5 *		x+																											
cgpGmo-S1456	2	49.7 *																													
cgpGmo-S1101a	2	49.7 *		x+																											
cgpGmo-S1068	2	49.7 *		x+																											
cgpGmo-S174	2	49.9 *																													
cgpGmo-S646	3	13.1																													

continued on the next page

Table S2 -continued

[illegible]

¹ For regional assessments AIC=All contemporary, Ioe=Iceland (all contemporary), GC=Greenland historical, GH=Greenland historical. For the cluster assessments I=Iceland inshore, E=East, W=West and N=Nuuk.

²DC=Distance to coastline, Lat=latitude, Long=longitude, MeB=Mean bottom temperature, MeS=Mean surface temperature, MIS=Minimum surface temperature, RST=Range surface temperature, and Sal=salinity

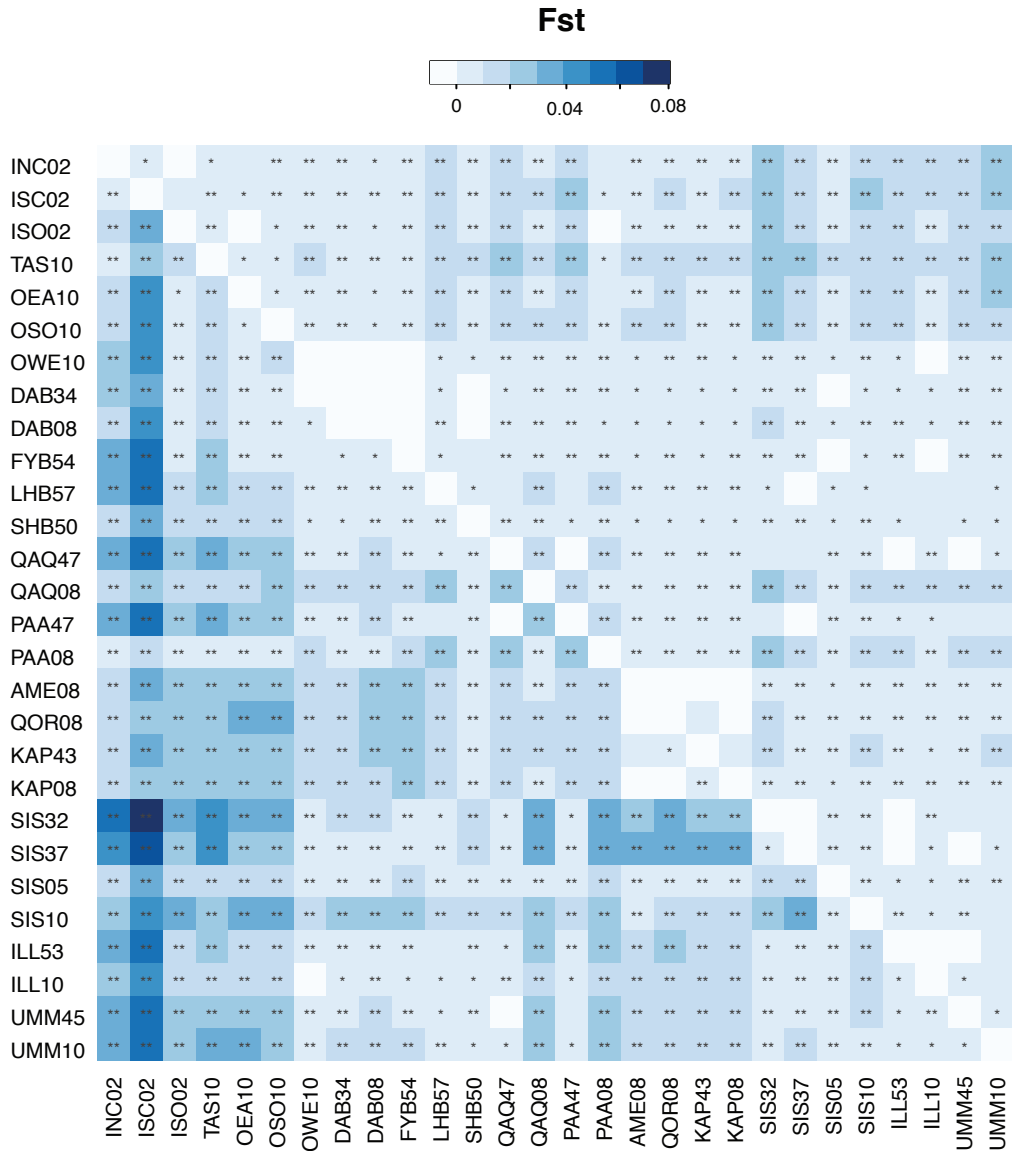


Fig. S1. Heatmap of pairwise F_{ST} values between samples. The lower left diagonal represents tests based on all loci while the upper right diagonal represents tests based on a subset of loci ($n=618$) excluding temporal and spatial outliers and loci in high LD. In both cases, comparisons that had significantly different allele frequencies at the $p<0.05$ level are marked by *, while differences that remained significant after FDR correction are marked by **. Samples are ordered according to hydrographic distance from the eastern-most sample.

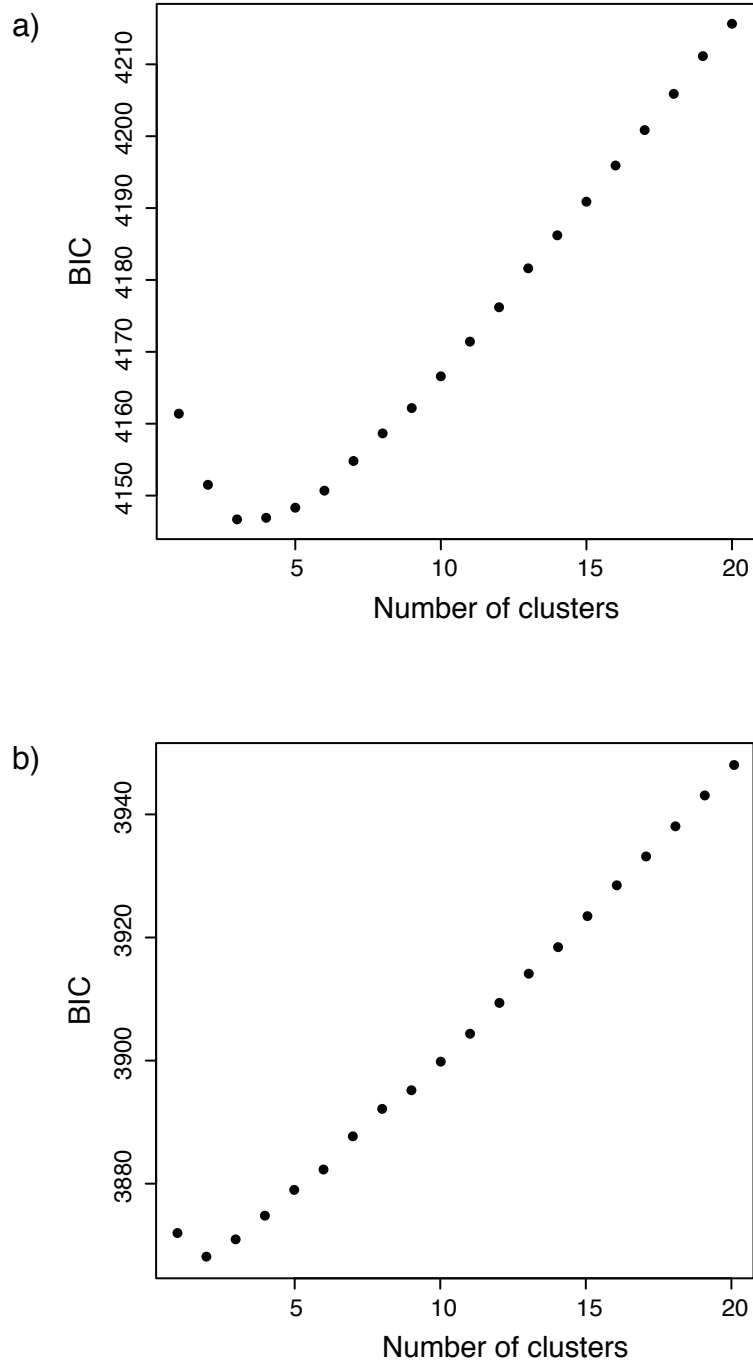


Fig. S2. Plot of the Bayesian Information Criterion (BIC) for clustering solutions with different numbers of clusters (K) based on all loci (a) and a subset of loci ($n=618$) excluding temporal and spatial outliers and loci in high LD (b).

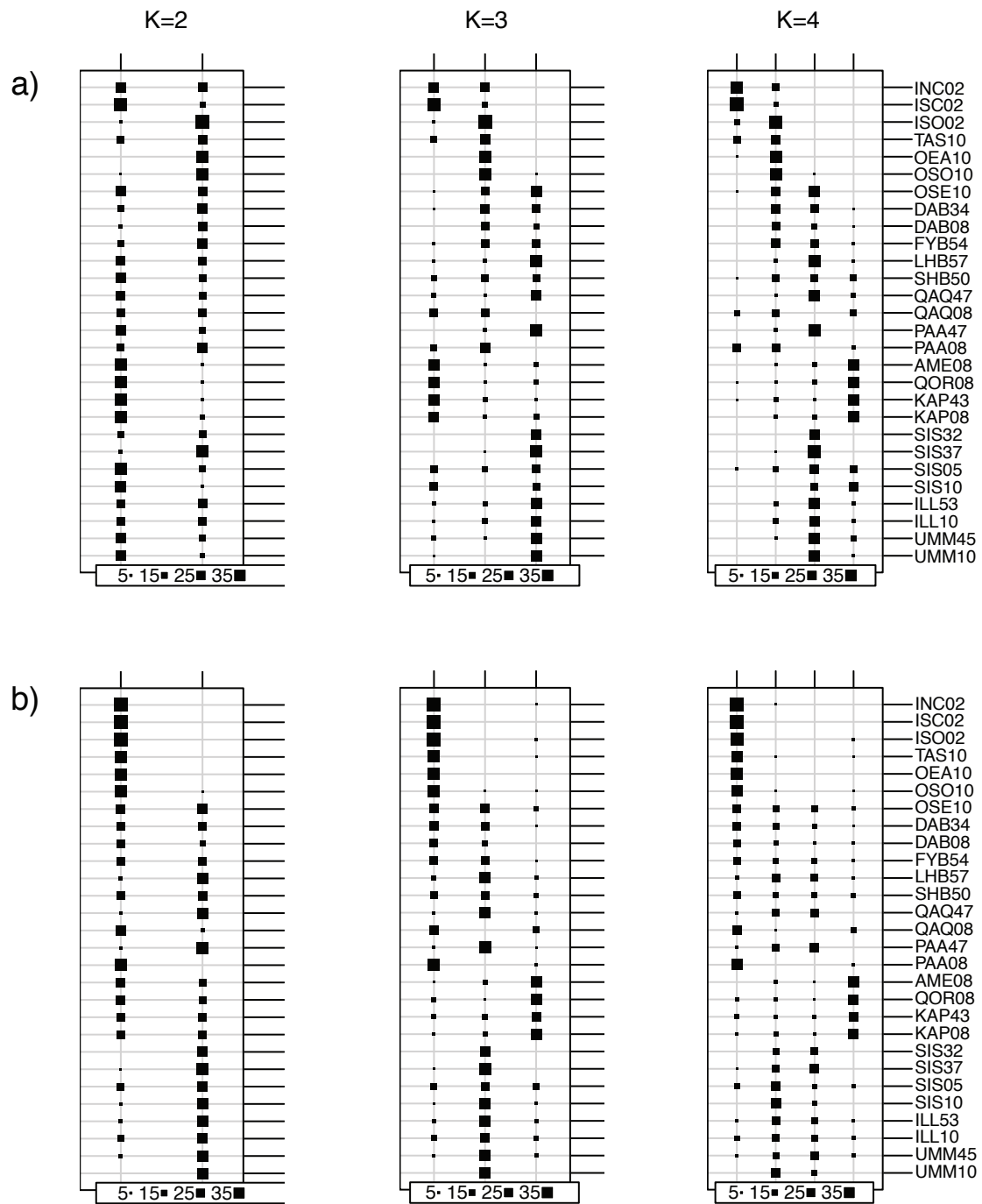


Fig. S3. Plots to illustrate the configuration of inferred clustering solutions for $K=2:4$ based on all loci (a) and a subset of loci ($n=618$) excluding temporal and spatial outliers and loci in high LD (b). Samples are ordered along the vertical axis according to hydrographic distance from the easternmost sample and the size of the black squares represent how many individuals from the sample were assigned to a given cluster.

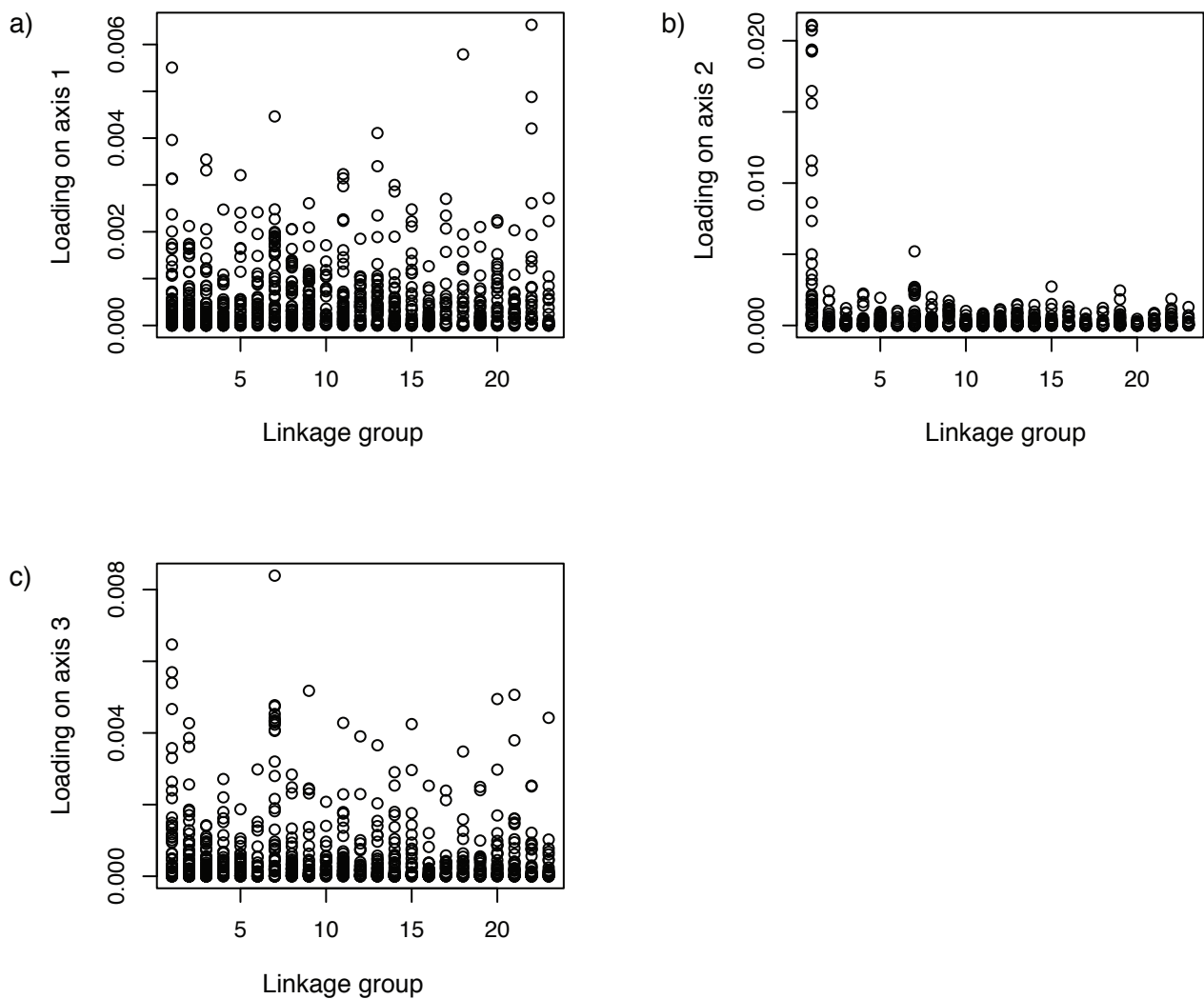


Fig S4. Loading plot representing the contributions of alleles from different linkage groups on the first (a), second (b), and third (c) discriminant function from the DAPC based on the four inferred clusters. Each dot represents an allele and only loadings involving SNPs placed on the linkage map (96% of the total panel; Table S1) are plotted.

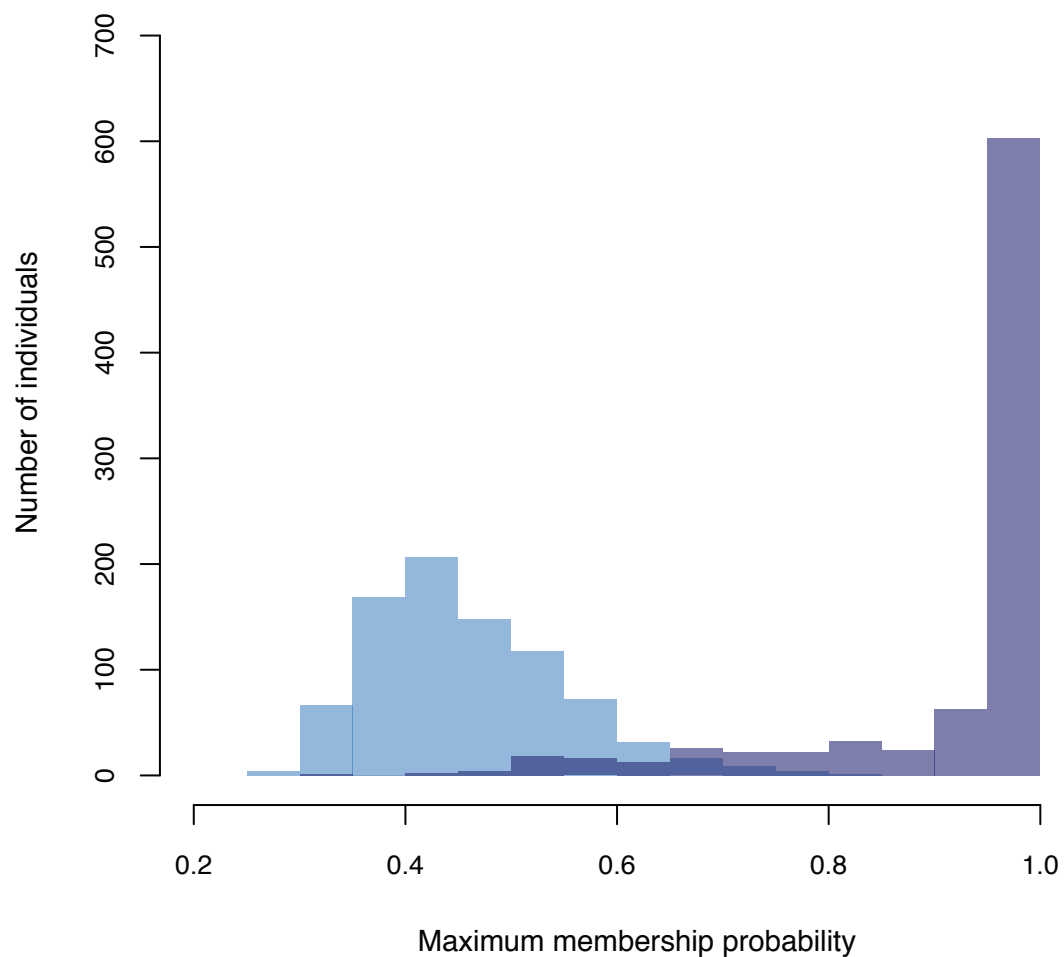


Fig. S5. Frequency histograms comparing the distribution of maximum individual posterior membership probabilities generated in the DAPC analysis based on clustering of the “pure samples” (see text; dark blue) and a DAPC analysis based on randomized prior cluster assignment (light blue).

CHAPTER 5

Evaluation of a high-throughput SNP genotyping platform for analysis of degraded DNA from historical fish samples

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ABSTRACT

DNA recovered from historical samples can generate unique insights into recent evolutionary history in natural populations. To fully harness the power of retrospective genetic analysis for elucidating patterns of both neutral and adaptive variation, there is, however, a need to identify high-throughput methods that will allow efficient genotyping at a genome-wide level in such studies. Challenges associated with degraded DNA quality have hampered the application of novel molecular sequencing and genotyping tools to historical samples, but certain methods are robust to suboptimal input material. Here, we show that the Illumina GoldenGate SNP genotyping platform can produce high-quality data with DNA samples extracted from archived fish otoliths up to 80 years old. Through analysis of 800 individuals (72 re-analyzed in independent assays) for 192-1536 SNPs, we found that genotype concordance between replicate sample pairs generally was high (>97%) for the majority of extracts and that call rates on average were >90%, except in the oldest available samples where it was about 76%. We found that low call rates and GenCall scores (an Illumina genotype quality metric) were associated with reduced concordance, and used this result to develop data quality filtering criteria that maximized data retention while limiting error rates. We hence demonstrate that the GoldenGate assay is a valuable method for efficient and reliable SNP genotyping in historical samples and lay out a flexible framework for evaluating genotyping quality to help ensure and document high data reproducibility in future studies.

Keywords: Historical DNA, SNP genotyping, temporal analysis, detecting selection, fish otoliths

INTRODUCTION

DNA recovered from historical samples can provide unique insights into recent evolutionary history by making it possible to track allele frequencies over time for direct retrospective assessment of genetic changes. Temporal genetic studies based on presumably neutral markers have been crucial for elucidating demographic processes in natural populations, e.g. through estimating effective population sizes, detecting loss of diversity, and assessing the stability of population structure and migration rates (see reviews by Wandeler (2007), Leonard (2008), and Nielsen and Hansen (2008)). However, there is also extensive – still largely untapped – potential to use historical samples to study the temporal dynamics of adaptive genetic variation (Hansen *et al.* 2012). In face of accelerated rates of global change, there is a growing need to improve our understanding of how quickly populations can adapt to altered environmental conditions, and historical DNA samples can make key contributions in this context. Until recently, however, temporal genetic studies were generally limited to studying selection at known candidate genes (e.g. Umina *et al.* 2005; Jensen *et al.* 2008; Marsden *et al.* 2012). Such efforts can provide important information in relation to specific hypotheses, but more comprehensive insights about how selection operates will require studies of temporal variation at a genome-wide level.

Rapid developments in sequencing and genotyping methods now offer unprecedented opportunities for screening large panels of genetic markers in population samples (Allendorf *et al.* 2010; Davey *et al.* 2011). Such data provide powerful tools for identifying signatures of selection in both space and time, but technical challenges associated with historical samples have hampered the application of high-throughput technologies in retrospective temporal studies. The DNA in historical samples is typically fragmented and only available in small quantities resulting in inadequate quality for many molecular methods. The ability to harness the power of genome-wide analysis for temporal studies therefore depends on identification of technologies that are robust to reduced sample quality and therefore can be used for reliable and efficient high-throughput genotyping in historical samples.

The Illumina GoldenGate Assay is a popular genotyping platform that allows for highly multiplexed genotyping of up to 3072 single nucleotide polymorphisms (SNPs) simultaneously. Based on hybridization of short (<60 bp) allele- and locus-specific probes to the template DNA, it should be a promising method for historical samples. Indeed, previous testing has demonstrated that performance can remain satisfactory with degraded DNA samples (Shen *et al.* 2005; Fan *et al.* 2006) and very low DNA concentrations (Campino *et al.* 2011). It has also been shown that both call rates and genotype concordance among replicates can remain very high with whole genome amplified (WGA) samples, although the results appear highly dependent on the quality and quantity of the initial DNA input (Hansen *et al.* 2007; Cunningham *et al.* 2008; Mead *et al.* 2008).

Here, we summarize our experience with the GoldenGate platform for SNP analysis of historical DNA samples extracted from archived fish otoliths up to 80 years old. We evaluate the overall genotyping performance and examine how different characteristics of both the DNA extracts and the applied SNP panel affect data quality. Because genotyping with historical samples is likely to be more error-prone than analysis of contemporary samples, it is important to apply stringent quality control measures (Bonin *et al.* 2004; Pompanon *et al.* 2005). Yet, at the same time, such measures are associated with a risk of discarding useful data. Therefore, a primary purpose of this work was to identify quality filtering criteria that would maximize data retention for analysis while limiting error rates. The presented results are useful for assessing the general reliability of Illumina GoldenGate SNP data generated from partially degraded DNA and for optimizing designs of future genotyping projects.

MATERIALS AND METHODS

SNP and sample selection

We analyzed historical samples of Atlantic cod *Gadus morhua* with a previously validated SNP panel specifically developed for this species and optimized for the GoldenGate platform (Hubert *et al.* 2010; Bowman *et al.* 2011). This assay has already been successfully applied to genotyping of cod population samples based on modern DNA (e.g. Bradbury *et al.* 2010; Nielsen *et al.* 2012), but it was uncertain how well it would work with suboptimal DNA extracts.

The source of historical DNA was archived otoliths from commercial fish stocks. Because of extensive archived collections, otoliths represent an unparalleled resource for retrospective genetic studies if the DNA can be recovered for high-throughput analysis. However, these samples are also associated with particular challenges, namely that 1) DNA can only be recovered from the surface of these calcareous structures and therefore it is not possible to decontaminate samples prior to DNA extraction, 2) the DNA is degraded from postmortem damage and 3) only a small amount of DNA can be recovered from each sample. The samples analyzed here originated from several different cod populations and had been collected between 1928 and 2002.

Due to the exploratory nature of this work, the genotyping was completed over multiple rounds, assaying different panels of SNPs as outlined in Table 1. To evaluate the reproducibility of results, a number of samples were genotyped in two or more independent assays allowing comparison of genotype calls among replicates (see Table 1).

DNA extraction, quantification and pre-screening

DNA was recovered from the surface of the otoliths using an Omega EZNA Tissue DNA kit (Omega Bio-Tek, USA) following the protocol described by Therikildsen *et al.* (2010). The DNA was eluted in a total volume of 150 μ l buffer. For a subset of samples, we quantified the DNA concentration with a Nanodrop 2000 spectrophotometer (Thermo Scientific, USA) and a Quant-iT dsDNA High-Sensitivity Assay Kit on a Qubit fluorometer (Invitrogen Life Technologies, USA). The Nanodrop measurements indicated that the concentration of the extracts ranged between 1-125 ng/ μ l (the majority <20 ng/ μ l) while the Qubit measurements, indicated that concentrations were much lower ranging between <0.1-37 ng/ μ l (mean of 4 ng/ μ l). Although there was some variation, the two types of concentration measurements for each sample were correlated ($\rho=0.88$, $p=0.0002$, Spearman Rank Correlation Test). The generally lower measurements obtained with the Qubit may most reliably reflect the DNA concentration because it is based on a fluorescent dye that only binds to the molecule of interest. Nanodrop measurements, on the other hand, are based on UV absorbance, which cannot distinguish between DNA, RNA, degraded nucleic acids, free nucleotides, and other contaminants, therefore probably overestimating DNA concentration.

A total of 5 μ l of each extract was used for the GoldenGate assay, so input quantities ranged from 0.5 to possibly 625 ng. For the majority of samples, the DNA input was thus much lower than the 250 ng recommended by Illumina. To more closely follow the recommendations, we tried to increase the DNA quantity with whole genome amplification (WGA). In batch 1 (see Table 1), we thus included 9 WGA products generated either with multiple displacement amplification using a REPLI-g kit (Qiagen) or with Sigma-Aldrich's GenomePlex method.

To pre-screen DNA extracts for cross-sample contamination, we amplified four highly polymorphic microsatellites (mean number of alleles 19) in all samples and removed individuals that amplified >2 alleles for any locus or that failed to produce reliable amplification within 2-3 attempts. The amplification was performed with a PCR multiplex kit (Qiagen, Germany) and we analyzed the

fragments on an ABI 3130 Genetic Analyzer (Applied Biosystems, USA) or a Basestation51 automated sequencer (MJ Research/Bio-Rad). All DNA extraction as well as WGA and PCR preparation with historical samples were conducted in an ancient DNA laboratory where no contemporary fish samples had been processed.

SNP Genotyping

The SNP genotyping was performed at Gen-Probe (formerly Tepnel Life Sciences), Scotland for batches 1 and 2 and at the Roslin Institute at the University of Edinburgh, Scotland for batches 3 and 4. In all cases, standard protocols for running GoldenGate assays were followed, but historical samples were kept separate from contemporary samples to minimize the risk of contamination.

The GoldenGate assay is based on hybridization of both locus-specific and allele-specific oligonucleotide probes to the template DNA. Following extension and ligation, a PCR step with fluorescently labeled primers ensures that matching templates are labeled with either Cy3 or Cy5, depending on the allele for the particular SNP, and the PCR products then are hybridized to specific beads on an array matrix. Genotypes for each SNP are called based on the observed signal intensities for the two fluorescent colors, as detected with an array scanner. A genotype that is homozygous for a SNP locus will display a signal in either the Cy3 or Cy5 channel, whereas a genotype that is heterozygous for this SNP will display signals in both channels. This way, each genotype is associated with a measure *Theta* that indicates the color of the signal (ranging from 0-1 where 0 is exclusively one color and 1 exclusively the other) and a measure *R* that indicates the overall signal intensity. When the normalized values for these variables are plotted for multiple samples, the data should form three distinct clusters corresponding to the three genotypes for successfully analyzed SNPs (see Fig. 1).

We used the GenomeStudio Software (Illumina Inc.) to cluster data and call genotypes. The automatic genotype cluster definitions based only on the historical data generated genotype calls for almost all data points, but resulted in low concordance among replicates (down to ~70% on average). Accordingly, we instead used manually edited cluster positions that had been adjusted to fit data from modern samples ("standard cluster"). While these may represent conservative definitions of where genotype clusters should be positioned, we observed slight shifts in intensity and *Theta* values for historical samples (see example in Fig. 1). Therefore, we further edited the clusters manually to best match the positions in the historical data ("fitted cluster").

Data quality evaluation

The quality of a final SNP data set depends both on how many data points are retained for analysis after quality control filtering and on the error rate. We therefore evaluated genotyping success based on two measures: the call rate (proportion of SNPs or samples genotyped) and the reproducibility measured as the concordance of genotypes called among sample replicates. We examined how a number of factors listed in Table 2 affected the call rate and genotyping concordance among replicates. Due to variation in the applied SNP panels, the quality was assessed for each data batch separately. Here, we present data mostly for batches 3 and 4, but conclusions were generally similar among all batches. The genotyping performance for contemporary samples was generally high (call rates for most samples exceeding 95% and genotyping concordance for replicated individuals typically >99%). Therefore, we only present data for the historical samples.

RESULTS AND DISCUSSION

Cluster set selection and call rates

The mean genotype concordance was high (>98%) for most replicate samples, although there was a clear tendency for reduced concordance in samples with low call rates (Fig. 2a). Genotypes called with cluster definitions fitted specifically to the historical data (“fitted cluster”) provided considerably higher call rates, and only marginally lower concordance compared to cluster definitions fitted to the distribution of data from modern samples (“standard cluster”; Fig. 2a). Since concordance remained high with the “fitted” cluster set, we used this for all further processing to maximize data inclusion.

Sample characteristics

In addition to showing reduced call rates, the few samples that did not exhibit high genotype concordance appeared to also be characterized by low GenCall (GC) scores (Fig. 2b). Especially in batch 4, which was characterized by generally lower scores than batch 3, there appears to be a threshold above which all replicated samples had high concordance, but below which replicate pairs showed reduced concordance. Fortunately, the majority of samples in both batch 3 and 4 showed GC scores above the inferred threshold (Fig. 2b). However, since the call rates and GC score varied between years and populations (Fig. 3), strict removal of all individuals with low average GC scores would make the resulting sampling scheme very uneven in terms of numbers of individuals retained. Hence, instead of removing entire individuals with low average GC scores, we removed single genotypes with GC scores below a threshold value. This eliminated the most uncertain data points (those that are located too far from the center of a cluster in plots of normalized R vs. normalized Theta (see Fig. 1)) and thereby considerably improved concordance, while keeping more reliable genotypes for problematic individuals, so that reasonable sample sizes could be maintained for all sampling years.

The tendency for older samples to have lower call rates and concordance (Fig 3) is expected because DNA degrades with time, so the observed pattern in fact validates the authenticity of the DNA samples (demonstrating that they are not contaminated with contemporary high quality DNA). Notably, however, there was considerable variation in sample performance between populations and years, indicating that other factors than sample age affect DNA quality (e.g. storage conditions, method of collection etc.).

Genotyping performance was somewhat correlated with DNA concentration, with all the poorer performing samples having very low concentration. Interestingly, however, many samples with extremely low DNA concentration did show high genotyping success (results not shown). Amplification success with microsatellites appeared to be a reliable predictor of genotyping success, with samples that adequately amplified in the initial PCR attempt and that required fewer PCRs to pass the pre-screening tests having considerably higher call rates. This indicates, that microsatellite amplification success is a useful predictor of high SNP genotyping success and can be used as a criterion for sample selection in future projects.

For two of the whole genome amplified samples (one from each of the tested methods), we observed genotyping success comparable to or marginally improved over genomic sample replicates (similar call rates and genotype concordance >98%). However, for the remainder of comparisons, the WGA products performed much poorer than genomic DNA replicates with substantially reduced call rates and concordance as low as 50%. Since there appeared to be no benefit, but considerable risk to using WGA samples here, we used unprocessed DNA extracts for all following assays. Given that we only tested the technique on a small number of samples here

and that WGA on degraded samples has been successful in other GoldenGate applications (Hansen *et al.* 2007; Mead *et al.* 2008), this technique may warrant further investigation for historical otolith samples.

SNP characteristics

Similar to comparisons among samples, there was a tendency for SNPs with higher call rates to show better concordance, although the relationship was not very strong (Fig. 4a). The 10th percentile GC score did not seem correlated with concordance among SNPs and neither did, in general, cluster separation, minor allele frequency, position of heterozygote cluster or signal intensity (results not shown). Therefore, neither of these variables warrant data filtering. While we saw slightly better performance of SNPs with a higher design scores, the differences were relatively small (Fig. 4b), so even SNPs with a suboptimal predicted probability of performing well in the GoldenGate Assay (designability rank 0.5) may work well with historical samples.

CONCLUSIONS

Our study showed that SNP genotyping of historical fish DNA samples with the Illumina GoldenGate method generally yielded good concordance among replicate sample pairs and call rates within acceptable limits, although not as high as for contemporary samples. The data quality assessment showed that sample call rate and sample GC scores appeared to correlate with data reproducibility, so filtering data based on these measures may reduce overall error rates. Individual SNP call rates also seemed to be loosely associated with variation in concordance, so could be applied as a filtering criterion. However, when replicate samples are analyzed, the mean observed genotype concordance for each SNP is probably a more informative indicator that can be used to exclude loci that yield the least reproducible genotypes.

Appropriate thresholds for filtering will depend on characteristics of each specific data set and will always reflect a balance between retaining as much data as possible for analysis and reducing error rates to an acceptable level. Based on the results presented here, and additional analysis, we adopted the following criteria:

- For batch 2 and 4: Only genotypes with a GC score > 0.4, SNPs with a concordance of >0.7, and samples and SNPs with a call rate >0.5 were included in the final data set.
- For batch 3: Only genotypes with a GC score > 0.25, SNPs with a concordance of >0.9, and samples and SNPs with a call rate >0.7 were included in the final data set.

The selection of different thresholds reflects that in the batch 2 and 4 data, a poorer genotype concordance required a stricter GC criterion. This removed the most uncertain data points across samples and SNPs resulting in lower overall call rates. In batch 3, the same conservative GC filtering was not necessary given the high, concordance among sample replicate pairs. Consequently, call rate and SNP concordance thresholds could then be set higher in order to remove only particularly problematic samples and loci.

With filtering criteria excluding a minimum of individuals from the final data sets, the mean concordance of SNP genotypes in replicate samples was >98% and the mean call rate 95% for the batch 3 study, while the batch 4 study showed 97% genotype concordance and a mean call rate >90% (for batch 2, including samples from 1928, the mean call rate was lower, about 76%, but genotype concordance similar to the other batches). These statistics demonstrate that the Illumina GoldenGate platform is well suited for genotyping historical samples and that the error rates in the resulting data should be low. It must be stressed, however, that DNA quality varies considerably

among historical samples, so it will be important to carefully assess each dataset. The approach presented here provides a flexible framework for evaluating genotype data obtained from historical samples to help ensure and document high data reproducibility in future studies.

ACKNOWLEDGEMENTS

We would like to thank Fisheries and Oceans Canada and the Greenland Institute of Natural Resources for supplying historical otolith samples. We are also grateful to Eske Willerslev and M. Thomas P. Gilbert at the Centre for GeoGenetics at the Natural History Museum of Denmark for generously providing access to ancient DNA laboratory facilities and to Rob Ogden, Richard Talbot and David Morrice helpful assistance with the SNP genotyping. Rob Ogden also performed some of the WGA reactions and Thomas Damm Als shared R-scripts for data analysis. The study received financial support from the European Commission, as part of the Specific Targeted Research Project Fisheries-induced Evolution (FinE, contract number SSP-2006-044276) and from the Danish Agency for Science, Technology and Innovation as part of the Greenland Climate Research Centre.

LITERATURE CITED

- Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetics. *Nature Reviews Genetics*, **11**, 697–709.
- Bonin A, Bellemain E, Bronken Eidesen P *et al.* (2004) How to track and assess genotyping errors in population genetics studies. *Molecular Ecology*, **13**, 3261–3273.
- Bowman S, Hubert S, Higgins B *et al.* (2011) An integrated approach to gene discovery and marker development in Atlantic cod (*Gadus morhua*). *Marine Biotechnology*, **13**, 242–255.
- Bradbury IR, Hubert S, Higgins B *et al.* (2010) Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **277**, 3725–3734.
- Campino S, Auburn S, Kivinen K *et al.* (2011) Population genetic analysis of *Plasmodium falciparum* parasites using a customized Illumina GoldenGate genotyping assay (JR Fitzgerald, Ed.). *PLoS ONE*, **6**, e20251.
- Cunningham JM, Sellers TA, Schildkraut JM *et al.* (2008) Performance of amplified DNA in an Illumina GoldenGate BeadArray Assay. *Cancer Epidemiology Biomarkers & Prevention*, **17**, 1781–1789.
- Davey JW, Hohenlohe PA, Etter PD *et al.* (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics*, **12**, 499–510.
- Fan J-B, Gunderson KL, Bibikova M *et al.* (2006) Illumina universal bead arrays. *Methods in enzymology*, **410**, 57–73.
- Hansen HM, Wiemels JL, Wensch M, Wiencke JK (2007) DNA quantification of whole genome amplified samples for genotyping on a multiplexed bead array platform. *Cancer Epidemiology Biomarkers & Prevention*, **16**, 1686–1690.
- Hansen MM, Olivieri I, Waller DM, Nielsen EE, The GeM Working Group (2012) Monitoring adaptive genetic responses to environmental change. *Molecular Ecology*, **21**, 1311–1329.
- Hubert S, Higgins B, Borza T, Bowman S (2010) Development of a SNP resource and a genetic linkage map for Atlantic cod (*Gadus morhua*). *BMC genomics*, **11**, 191.
- Jensen LF, Hansen MM, Mensberg KLD, Loeschcke V (2008) Spatially and temporally fluctuating selection at non-MHC immune genes: evidence from TAP polymorphism in populations of brown trout (*Salmo trutta*, L.). *Heredity*, **100**, 79–91.
- Leonard JA (2008) Ancient DNA applications for wildlife conservation. *Molecular Ecology*, **17**, 4186–4196.
- Marsden CD, Woodroffe R, Mills MGL *et al.* (2012) Spatial and temporal patterns of neutral and adaptive genetic variation in the endangered African wild dog (*Lycaon pictus*). *Molecular Ecology*, **21**, 1379–1393.
- Mead S, Poulter M, Beck J *et al.* (2008) Successful amplification of degraded DNA for use with high-throughput SNP genotyping platforms. *Human Mutation*, **29**, 1452–1458.
- Nielsen EE, Hansen MM (2008) Waking the dead: the value of population genetic analyses of historical samples. *Fish and Fisheries*, **9**, 450–461.
- Nielsen EE, Cariani A, Aoidh EM *et al.* (2012) Gene-associated markers provide tools for tackling illegal fishing and false

- eco-certification. *Nature communications*, **3**, 851.
- Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes, consequences and solutions. *Nature Reviews Genetics*, **6**, 847–846.
- Shen R, Fan J-B, Campbell D *et al.* (2005) High-throughput SNP genotyping on universal bead arrays. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **573**, 70–82.
- Therkildsen NO, Nielsen EE, Hüsey K, Meldrup D, Geffen AJ (2010) Does DNA extraction affect the physical and chemical composition of historical cod (*Gadus morhua*) otoliths? *ICES Journal of Marine Science*, **67**, 1251–1259.
- Umina PA, Weeks AR, Kearney MR, McKechnie SW, Hoffmann AA (2005) A rapid shift in a classic clinal pattern in *Drosophila* reflecting climate change. *Science*, **308**, 691–693.
- Wandeler P, Hoeck PEA, Keller LF (2007) Back to the future: museum specimens in population genetics. *Trends in Ecology & Evolution*, **22**, 634–642.

FIGURE LEGENDS

Fig. 1. GenomeStudio plot for a single SNP showing the normalized signal intensity (Norm R) vs. the signal color (Norm Θ) measured for each sample (illustrated by dots). The ellipses represent the cluster positions that define genotype calling (only samples within a certain distance from the cluster center get called). For this particular SNP (a) shows the “standard” cluster position fitted to modern samples from a previous run (not plotted here), and (b) shows “fitted” cluster positions adjusted to the historical samples plotted here.

Fig. 2. Mean genotype concordance plotted against (a) mean call rate for each replicate sample pair based on genotypes called with the “standard” (yellow) or the “fitted” (green) cluster definition sets in batch 4 and (b) mean sample 10% GC score in batch 3 (blue) and batch 4 (pink).

Fig. 3. Mean (dots) and ± 1 standard deviation (error bars) of sample call rates (a) and mean concordance among replicate pairs (b) for samples collected in different years and genotyped in batch 2 (purple), batch 3 (blue), and batch 4 (pink). For reference, six contemporary replicate samples genotyped with batch 4 have been added.

Fig. 4. Mean concordance (proportion of replicate sample pairs that showed identical genotypes) plotted against the call rate for each SNP (a) and mean SNP call rate plotted against the SNP designability score (b). Data from batch 3 is plotted in blue while data from batch four is plotted in pink.

AUTHOR CONTRIBUTIONS BOX

NOT and EEN designed the research. NOT and JHH performed the research. NOT analyzed the data and wrote the paper with input from the other authors.

Table 1. Overview of the number of samples, sample replicates, collection years, and the number of SNPs assayed and successfully clustered (genotypes called) in each batch

Batch	Samples	Replicate samples	Collection years	SNPs assayed	SNPs clustered
1	17	13 (4 identical extracts, 9 WGA samples)	1928-1976	1536	1282
2	73	10 (all in three repeat assays)	1928-1965	1536	1282
3	340	27 (1 modern)	1931-1954	1152	1011
4	370	23 (6 modern)	1960-2002	192	166

Table 2. Overview of the data-, sample- and SNP characteristics considered in evaluation of genotyping performance

Type	Variable	Description
Data	Genotype clustering set	A set of cluster definitions that determine the genotype calling as visualized in plots of signal intensity (<i>R</i>) vs signal color (<i>Theta</i>) for each data point (SNP/sample combination).
	GenCall score (GC)	An Illumina quality metric ranging from 0-1 indicating the reliability of called genotypes. Each data point is given a score based on how well the genotype clusters are separated, how far the datapoint is located from the center of a cluster and on the intensity of the signal.
Samples	Sample genotyping concordance	The number of SNPs with identical genotype calls in repeat assays (with the same DNA extract) divided by the total number of SNPs that were successfully genotyped in both assays
	Sample call rate	The number of genotypes called for a sample divided by the total number of SNP successfully genotyped
	Sample 10% GC score	The 10th percentile rank of GenCall scores (one for each SNP) for the sample
	Sample age	Years since the sample was collected
	DNA concentration	DNA concentration quantified with a Nanodrop spectrophotometer or Qubit fluorometer
	Microsatellite amplification success	Classification of the initial microsatellite amplification success to "good", "excessive stutter", "poor" or "failed" as indicative of DNA quantity and/or quality in the sample extract
SNPs	SNP genotyping concordance	The number of sample replicate pairs that gave identical genotype calls in repeat assays divided by the number of replicate pairs for which the SNP was successfully genotyped in both assays
	SNP call rate	The number of samples for which a genotype is called for the SNP divided by the total number of samples
	SNP 10% GC score	The 10th percentile rank of GenCall scores (one for each sample) for the SNP
	Cluster separation	A measure ranging from 0-1 of how well genotype clusters are separated for a SNP
	Minor allele frequency	The frequency of the rarest allele among the called genotypes for a SNP
	AB T Mean	The mean normalized theta values of the heterozygote cluster for the SNP. This metric indicates how well the heterozygote cluster is separated from the homozygotes
	AB R	The mean normalized intensity (<i>R</i>) of the heterozygote cluster. This metric helps identify SNPs with low intensity data
	SNP design score	A metric calculated through an Illumina proprietary algorithm based on the SNP flanking sequence to indicate the probability of a SNP performing well in the GoldenGate assay

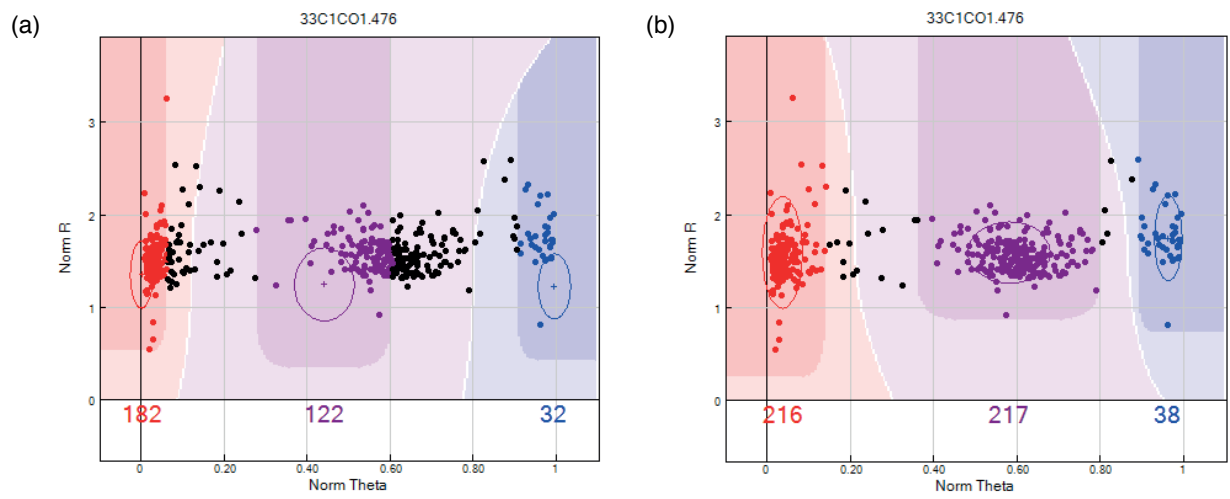


Fig. 1

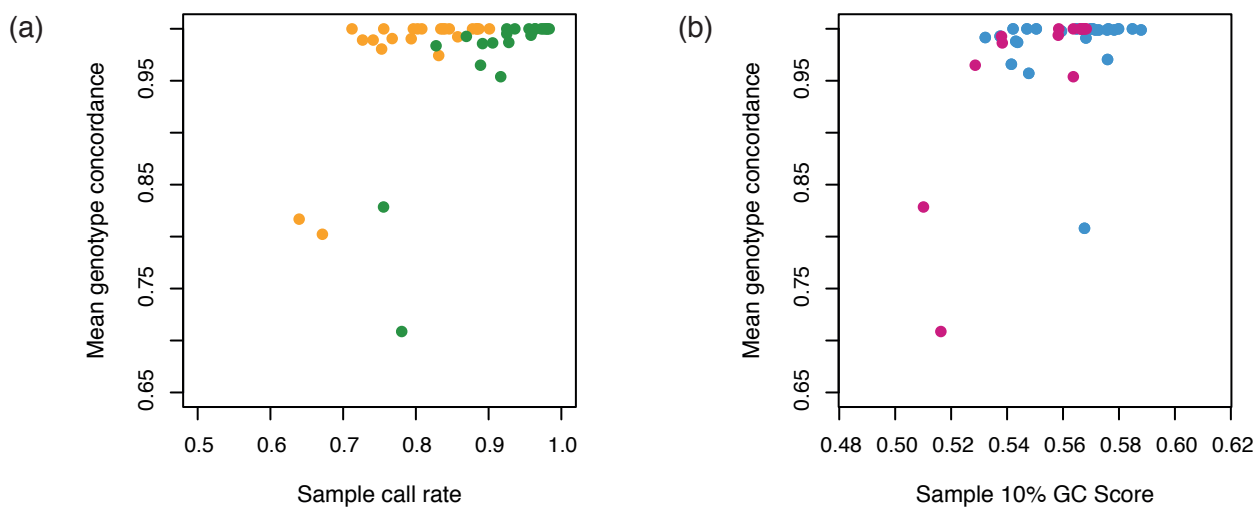


Fig. 2

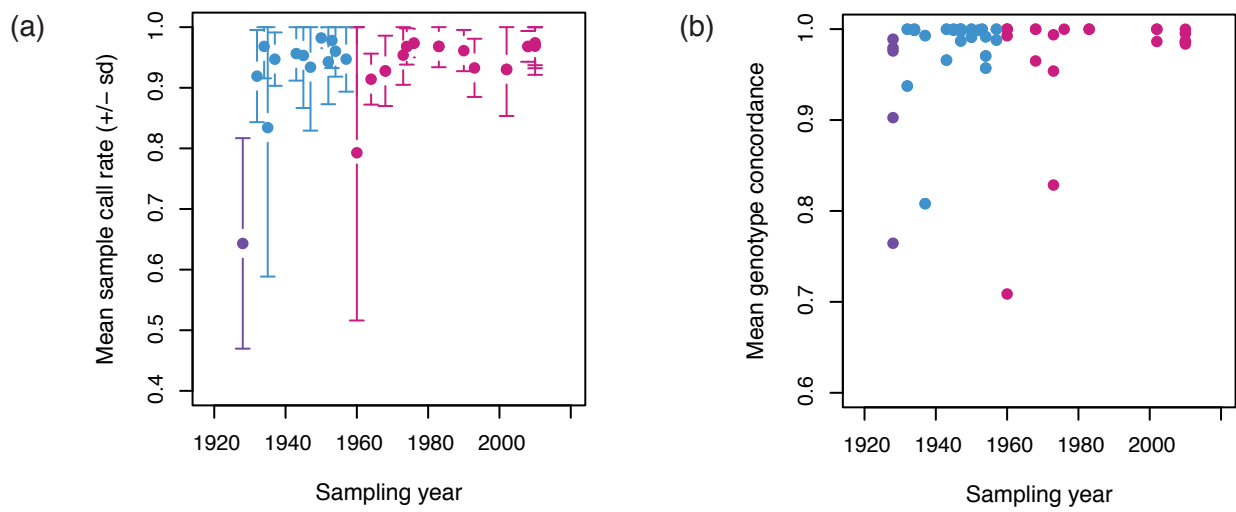


Fig. 3

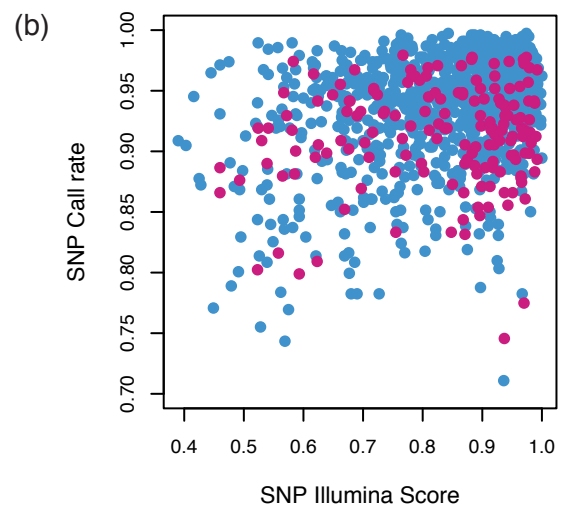
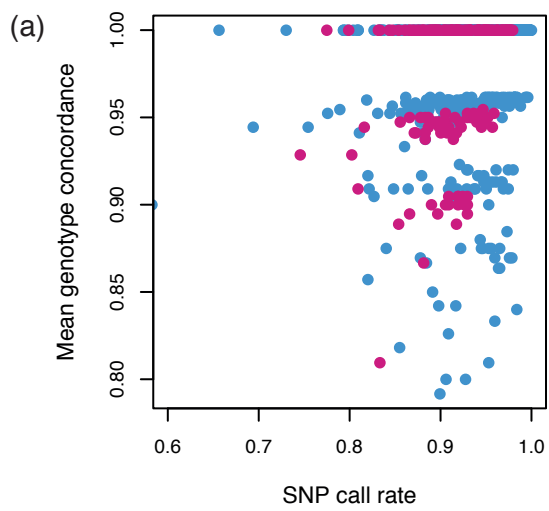


Fig. 4

CHAPTER 6

Ftemp: a method to detect genomic signatures of selection from temporal sampling

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ABSTRACT

Genome scan approaches are frequently applied for identifying genomic regions under selection. Methodological developments have generally focused on applications to spatial variation among different populations. However, the principle is equally applicable to genetic variation in samples collected over time from a single population. Such temporal data provide powerful insights about evolution in 'real time', but neutral expectations in temporal scenarios may be different from expectations generated through spatial models. Thus, if spatial genome scan methods are applied to temporal data, it is unclear both how to parameterize simulations and how to interpret results. Targeted methods are therefore needed. Here, we propose a framework that compares the observed temporal differentiation at individual loci to a neutral distribution generated through simulations of drift within a single population, which is a modification of a commonly applied spatial genome scan approach. Application to example datasets shows that it identifies a greater number of temporal outliers than methods designed to evaluate spatial patterns, but that the strongest outliers are consistently identified by all methods. We also demonstrate that our model is generally robust to uncertainty of parameter input values and that it responds as expected to variations in sampling configuration. The main strength of our proposed approach is that it provides an intuitive and simple framework that generates readily interpretable results for temporal data.

Keywords: outlier test, genome scan, temporal, selection, single nucleotide polymorphisms (SNPs)

INTRODUCTION

Since evolution is genetic change over time, comparisons of temporally spaced DNA samples should offer the most direct way to study its fine-scale dynamics. Temporal genetic data can be generated from targeted repeat sampling of wild or laboratory populations or it can be based on archived, recoverable or resurrectable historical material or results presented in previous studies for retrospective assessment. Regardless of the data source, the possibility to track allele frequencies in 'real time' provides unique insights into the evolutionary changes over the studied time period.

A key component of understanding evolutionary change is to disentangle the effects of ongoing selection from the effects of demography, gene flow, and population history. Generally, selection is expected to act only on specific loci and closely linked sites, whereas neutral forces like drift and migration are expected to exert relatively uniform effects across the entire genome (Cavalli-Sforza 1966). These interacting processes will shape genetic variation in both space and time and hence, loci that show divergent patterns of differentiation compared to the rest of the genome either between populations or between time points within a single population are likely affected by selection (Lewontin & Krakauer 1973). This principle forms the basis of population genomics or genome scan approaches, which are widely applied to identify genomic regions undergoing selection (Luikart *et al.* 2003; Storz 2005; Stinchcombe & Hoekstra 2007). Advances in the statistical framework underlying these methods have primarily focused on contemporary spatial variation between populations (e.g. Beaumont & Nichols 1996; Foll & Gaggiotti 2008; Excoffier *et al.* 2009), probably due to the much greater abundance of such data sets. Limited by sample availability and technical constraints associated with degraded DNA in historical samples, temporal data sets have been more difficult to generate. However, as entire genomes of ancient specimen are now being sequenced (Rasmussen *et al.* 2010), we are likely to see an increase both in the genomic coverage and time scales considered in temporal data sets, and therefore there is a need to carefully evaluate the statistical methods applied for their analysis.

The first formalized test of the principle that selection should cause increased variance in allele frequencies at specific loci compared to expectations under neutrality was proposed by Lewontin and Krakauer (1973). Based on theoretical predictions about the distribution of single-locus F_{ST} estimates, this test has been severely criticized, but primarily for its application to spatial genetic variation (e.g. Nei & Maruyama 1975; Robertson 1975). It should be much more robust in applications to temporal data from a single population (Lewontin & Krakauer 1975; Hedrick *et al.* 1976; Gaines & Whittam 1980). Nevertheless, the analytical deductions inherent to the test are based on approximations to a chi-square distribution, which may not generally provide an accurate description of the distribution of temporal allele frequency variance among loci (Mueller *et al.* 1985). Simulations have demonstrated that although the approximation is reasonable for a broad set of parameter values, substantial deviations arise under a variety of conditions (Waples 1989b; Goldringer & Bataillon 2004). With the computational power available today, it may thus be preferable to base tests on direct simulations rather than theoretical approximations.

A number of alternative tests have been proposed to evaluate whether observed temporal allele frequency variation can be explained by drift and sampling alone (e.g. Fisher & Ford 1947; Watterson 1982; Mueller *et al.* 1985; Waples 1989a). Yet, none of these have been widely adopted, perhaps because they were developed for specific purposes, have been difficult to implement, or have also relied on similar chi-square approximations. More recently, Goldringer and Bataillon (2004) suggested a generalized simulation-based test for homogeneity of locus-specific temporal variance in allele frequencies, but it was only formulated for comparisons of two temporally spaced samples. Sandoval-Castellanos (2010) developed a Bayesian simulation method that can incorporate samples from multiple time points and Bollback (2008) proposed a method to simultaneously estimate the effective population size (N_e) and the selection coefficient,

also from serial sampling of the same population. Common to these three methods, however, is the need to generate separate sets of simulations/computations for every locus—a task that becomes increasingly cumbersome as the panels of studied genetic markers get larger.

In the absence of integrated multi-locus methods directed for samples collected over time, the first large-scale temporal genome scans that are now beginning to appear in the literature generally apply conventional methods designed for spatial sampling (e.g. Hansen *et al.* 2010; Poulsen *et al.* 2011; Bourret *et al.* 2011; Orsini *et al.* 2012). Because these methods fit models to the observed data, they will almost certainly detect major departures from genome-wide patterns regardless of the underlying assumptions about neutral expectations. However, the specific p-values or posterior probabilities for a locus being a significant outlier will only apply in relation to the inherent model scenario.

Spatial genome scan methods assume that samples are collected at a single time from geographically separated populations that exchange migrants. This is obviously different from serial sampling of a single population because samples here are much less independent. One of the most commonly applied spatial genome scan methods, BayeScan, is based on the multinomial Dirichlet likelihood to estimate locus- and population-specific effects from the observed variation in allele frequencies (Foll & Gaggiotti 2008). While this likelihood function should arise in a range of demographic equilibrium models (Balding 2003; Beaumont & Balding 2004), it has not been demonstrated to be valid for differentiation over time within the same population. Similarly, a second popular spatial method, *fdist* as e.g. implemented in the software Lositan (Antao *et al.* 2008) is based on comparing the observed differentiation among samples to an expected neutral distribution generated through coalescent simulations under an island model (Beaumont & Nichols 1996). Although the expected distribution of F_{ST} appears robust to some departures from this underlying demographic model (Beaumont & Nichols 1996), the degree to which the distribution under a temporal model would deviate from this standard has not been thoroughly evaluated. Therefore, although spatial genome scan methods may provide roughly accurate estimates for temporal data, the clear violation of basic model assumptions makes interpretation of the results difficult and could lead to yet unexplored over- or underestimation of the number of loci that are affected by selection over time. Consequently, there is a need for methods that are specifically tailored to temporal data.

We here illustrate how the spatial method proposed by Beaumont and Nichols (1996) can be adapted to meet this need. As mentioned above, the original formulation of this method compares the observed differentiation at each locus to a simulated null distribution generated under an island model. However, the approach is flexible because the null distribution can in principle be generated under any model and therefore be tailored to match a variety of scenarios. We propose that a simple model of drift within an isolated population can be a useful null expectation for analysis of data collected over multiple generations and we evaluate its sensitivity to variations in sampling configuration and uncertainty of input parameter values. To demonstrate how this model can be used to make intuitive inference from temporal data, we apply the method to two example data sets and compare its performance to that of two spatial genome scan methods.

MATERIALS AND METHODS

The model

Our approach is based on a forward Wright-Fisher (WF) model to evaluate how much variance in allele frequencies would be expected from drift and sampling error alone within an isolated population sampled over multiple generations. The effects of new mutations are ignored because the model is intended for data collected over time scales that are short enough for the effects of

mutations to be assumed negligible compared to other evolutionary forces. Migration can have contrasting effects on allele frequencies within a population over such time scales depending on the level of differentiation between the source and the recipient populations (Wang & Whitlock 2003; Fraser *et al.* 2007). These effects can be complex to generalize and can only be quantified with detailed data on source populations, which is often not available. Consequently, migration is also not explicitly included in this null model. Any loci that show greater differentiation over time than expected from simulations under the null model can therefore be affected either by selection or gene flow. Because selection is expected to only affect certain locations in the genome, whereas gene flow is expected to have genome-wide effects, the proportion of loci that significantly deviate from null expectations may provide insight into the importance of the two forces. However, complete disentanglement of these effects may require additional sampling from potential migrant source populations.

Each realization of the null model simulations is based on a single bi-allelic locus at initial frequency f_0 in a WF population of constant size N_e that reproduces over t_{max} generations. At each t_s generation, a sample of size n individuals is collected. The model should be parameterized to most closely match the sampling scenario of the observed data.

The initial allele frequency f_0 at each simulated locus is a random number between 0 and 1, but to generate a roughly uniform distribution of H_e values among the simulated loci, we enrich the simulations for low starting frequencies. The N_e for the study population should be estimated either based on the temporal allele frequency data (e.g. Wang 2005; Luikart *et al.* 2010), from single-sample genetic estimators (e.g. Waples & Do 2008; Wang 2009), or with demographic methods (e.g. Caballero 1994; Engen *et al.* 2007). Since inclusion of loci under selection can downward bias N_e estimates (Wang 2005; Palstra & Ruzzante 2008), N_e estimation from the genetic data should be performed iteratively, first identifying outlier loci from simulations with an N_e estimate based on all loci, and then re-estimating the N_e with those loci excluded and using this estimate for final simulations of the null distribution.

The generation time of the studied population needs to be estimated to convert sampling years into sampled generations. Since the model is based on forward simulations, the oldest sample is considered generation 0 and subsequent sampling years rounded to progressive generations, denoted t_s . The t_{max} is the generation where the contemporary sample was collected. The sample size n should be the harmonic mean number of individuals included in each sample. This way, for each simulated locus, we obtain a time series of allele frequencies corresponding to the sampling pattern in the real data.

For each locus, we quantify the temporal variance in allele frequencies between all samples in both observed and simulated data with Wright's F (Wright 1951) corrected for sampling effects following (Waples 1998):

$$F_{temp} = \frac{\text{var}(p)}{\bar{p}(1 - \bar{p})} - \frac{1}{2n}$$

where $\text{var}(p)$ is the variance in observed allele frequencies among time points (since this implementation of the model is for bi-allelic loci, we only need to keep track of one allele), \bar{p} is the mean observed allele frequency among time points and n is the harmonic mean number of individuals included in each sample.

The correction for sampling effects is important because missing data can make the actual sample size vary between loci in the observed data. Following the approach of Beaumont and Nichols (1996), we plot the differentiation (F_{temp}) as a function of the mean expected heterozygosity among samples (H_s) for each observed locus. We then simulate 100,000 independent loci and for each compute the F_{temp} and H_s . As in the fdist method, the paired values are rank-ordered by H_s and

grouped into overlapping bins of 4,000 points centered on every 2,000th point. For each H_s bin, we compute the quantiles of the distribution of F_{temp} values that define the confidence envelopes in which 95% and 99%, respectively, of the data points are expected to lie if behaving according to the model. To assess the statistical significance of departures from the neutral expectation, we compute empirical p-values for each locus as the proportion of simulated data points within its bin that show higher F_{temp} than the observed value. To correct for multiple testing, we compute the expected false discovery rate (FDR), or q-value, for each locus based on the distribution of p-values using the R-package qvalue (Storey & Tibshirani 2003). We considered tests significant when the FDR was <5% ($q < 0.05$).

All simulations and computations were completed with custom R-scripts that are available upon request.

Sensitivity analysis

Intuitively we expect that a larger number of sampling times t_s and larger sample sizes n for each sampling point would reduce the variance in allele frequencies and therefore F_{temp} among the simulated loci, but to explore how strong this effect would be, we compared the 95% confidence envelope in simulations based on different configurations. For this, we assumed a population of effective size 500 sampled a various number of times over 12 generations with variable sample sizes (see Table 1).

Because the input parameters that are needed to fit the model to the actual data from the studied population— N_e and generation length—are notoriously difficult to estimate in wild populations, we also evaluated the sensitivity of the method to uncertainties in these inputs. To assess the sensitivity to variations in N_e , we computed the 95% confidence envelope for different populations with N_e 's ranging from 50-50000, all sampled 4 times over 12 generations (generation 0, 4, 8, and 12) with sample sizes n of 30.

A simplifying assumption of the simulations is that the N_e remains constant over the study period. This is unlikely to be true in nature, so we assessed the sensitivity to random fluctuations in N_e in a separate set of simulations. Here, the N_e for each generation was derived by multiplying the N_e from the previous generation with a factor drawn randomly from a normal distribution with mean=1 and standard deviation either 0.25 or 0.5. Similarly, we also assessed the effect of a linear decrease of either 10 or 20% each generation over the 12 generations. The effect of uncertainty in generation length was assessed by comparing the 95% confidence envelope obtained from sampling generations 0, 4, 8, and 12 with simulations where the four samples had been collected 1-2 generations earlier or later (see Table 1).

Example application and comparison to spatial methods

To illustrate an example application, we analyzed two datasets from a previous study (Chapter 2) with the F_{temp} method. The data originates from a population of Atlantic cod (*Gadus morhua*) in the southern Gulf of St Lawrence, Canada, that had initially been sampled at the beginning and end of a 12 generation time interval and genotyped for 1047 SNPs (dataset 1). In a follow-up study, it was sampled 5 times over a 6-generation period (generations 0, 1, 2, 3, and 6), and these samples were genotyped for 160 SNPs (dataset 2). Because the selection of SNP panels was not independent, the two datasets were analyzed separately. Attempts to estimate the N_e for this population indicated that it was too large to get a point estimate from the genetic data (Therkildsen *et al.* 2010 and Chapter X). However, the lower 95% confidence limit on the N_e estimate was 568, so as a conservative measure, we used this as input in the simulations. The 95% confidence

envelope and the number of loci significant after FDR correction with the F_{temp} method were compared with analyses conducted on the same datasets with two popular methods for spatial genome scans. The first was Bayescan (Foll & Gaggiotti 2008), for which we used the default parameters, but varied the prior odds in favor of the model without selection between 10 and 3. The second was the standard spatial fdist method as implemented in the software Lositan (Antao *et al.* 2008) where we used the infinite alleles model and assumed 30 demes. For both programs, we used an FDR cut-off of 0.05 to identify significant outliers.

RESULTS

Sensitivity

As expected, both a higher number of sampling times (t_s) and larger sample sizes (n) reduced the temporal variance in allele frequencies, thereby narrowing the distribution of F_{temp} (Fig. 1). When sampling only the end points of the time period or when using very small sample sizes ($n=15$), the 0.975 quantile of the F_{temp} distribution was reduced for low heterozygosities (Fig. 1). For the remainder of scenarios, the distribution of F_{temp} was relatively constant across heterozygosities (except when approaching allele fixation at the extremes) and alteration of the simulation configurations did not cause major changes to the shape of the conditional distribution of F_{temp} (Fig. 1). Variations in the sampling scheme did notably affect the width of this distribution, however, indicating—unsurprisingly—that within the parameter space explored here, increased sampling effort leads to increased power to detect outlier loci that show divergent patterns of differentiation compared to null expectations.

The simulated distribution was relatively robust to uncertainty in estimation of the generation length. Sampling the population in earlier generations lead to a slightly narrower distribution than sampling of later generations after more drift had occurred in the population (Fig. 2). However, the effects were small even across the three-fold difference in generation length, indicating that uncertainty in estimating this parameter from wild populations will not heavily affect model expectations unless the N_e is very small.

The null distribution was very sensitive to small effective population sizes, however. With an N_e of 50, the distribution of F_{temp} was markedly wider than observed under any other scenario. Increasing N_e 's up to around 500 narrowed the distribution, but for N_e 's above this threshold, its exact magnitude had little impact on the simulated distribution with the sample sizes tested here (Fig. 2c). The distribution was also robust to fluctuations in N_e between generations (Fig. 2b). Only one of the demographic changes assessed here affected the conditional F_{temp} distribution, and that was the linear decrease by 20% every generation for 12 generations that reduced the quantile of F_{temp} at low heterozygosities. This resulted primarily from the low N_e at late generations (here we started with $N_e = 500$ so at generation 12 the N_e was 34) and the same effect was not observed for larger starting N_e 's (results not shown).

Example application and comparison to spatial methods

The simulated F_{temp} null distributions for the two example data sets provided a good fit to the observed data with 47.6 and 53.3% of data points above the median, respectively. In the two-sample comparison, 50 of the 1047 loci fell above the 97.5% quantile of the null distribution of F_{temp} and 10 of these were significant outliers following FDR control (Fig. 3a). In the five-sample comparison, 10 of the 160 loci were above the 0.975 quantile and 7 of these significant after FDR control (Fig. 3c). Using point estimates instead of lower 95% confidence limits on estimates of N_e

did not change the number of outliers and neither did changing the generation length estimate by +/- two years.

Analysis of the same data sets with the spatial model implemented in Lositan generated markedly wider null distributions, and consequently fewer significant outliers (Fig. 3 b and d). Yet, in the two-sample comparison, the null distribution generated by the spatial model at very low heterozygosities was much narrower than for the temporal model. Even the 0.995 quantile of the spatial model expectation excluded many loci that appeared to just be at the tail of the distribution of F_{st} values and not particularly divergent, likely leading to a high frequency of false outliers when applying the Lositan model to this dataset.

Although more outliers were detected with the Ftemp compared to the Lositan model, the significant Lositan outliers were all a subset of the Ftemp outliers. BayeScan detected even fewer outliers, but reassuringly, there was again complete overlap between these and the outliers from the other methods. With less conservative prior odds, BayeScan also detected more of the additional outliers (results not shown).

The three methods that are based on different models and assumptions thus provide results that roughly are qualitatively consistent for these example data sets. For practical purposes, the difference in the number of outliers detected results primarily from varying cut-offs for significance, with the Ftemp method applying the lowest threshold.

DISCUSSION

We have presented a model that can be implemented in an already established analytical framework for the purpose of detecting loci under selection from temporal genetic data. The simulated distributions changed with model configurations as expected, but generally showed robustness to variation in parameter input values, except for very small values of N_e . This indicates that the model should be useful for detecting loci affected by ongoing selection in serial samples from real populations.

With the Ftemp model, we detected a higher number of outlier loci than with either of two commonly applied genome scan methods designed for spatial samples, although the loci that showed the strongest differentiation were identified by all methods. The incongruence of results from different methods is not unique to temporal data as it has also often been reported in analyses of spatial data (e.g. Beaumont & Balding 2004; Nunes *et al.* 2010), highlighting the need to consider the risk of both type I and II errors. In principle, analysis of simulated data could reveal whether the higher number of outliers detected with the Ftemp method compared to results from spatial methods reflected greater power to detect selection in temporal data or elevated levels of false positives. Such simulation-based evaluation has been applied for comparison of spatial outlier detection methods (Pérez-Figueroa *et al.* 2010; Narum & Hess 2011), but findings will always depend on the model chosen to simulate selection. Since selection can act in many different ways, it is not certain how generally applicable conclusions from such evaluations are. In face of uncertainty about error rates it thus seems of primary importance that results are easily interpretable in relation to the study scenario for evaluation of reliability.

With the Ftemp model, the p-value obtained for each locus represents the probability that temporal differentiation of the magnitude observed (or larger) could arise due to drift and sampling alone in an ideal population of the same effective population size and generation length. For the Lositan model, p-values have no obvious meaning in relation to temporal change. For example, both the width of the null distribution and the number of FDR-corrected significant outliers varied somewhat depending on the number of demes that were used in the simulations under the island model

(results not shown). As this parameter has no clear relation to temporal variation within a single population, it becomes unclear how to parameterize the model. Consequently, the Lositan method, as well as BayeScan, probably will adequately identify loci that show much greater differentiation than the genome average in temporal data, but the threshold for significance is much more arbitrary than for analysis of spatial data. Further, although a full exploration of how the distribution of single-locus differentiation varies between temporal and spatial models is beyond the scope of this paper, the high number of low heterozygosity outliers observed with the Lositan analysis of the two-sample comparison suggest that spatial models may not always provide a satisfactory fit to temporal data throughout parameter space.

The model presented here clearly matches the sampling scenario for temporal data much better than spatial models do; however, it still includes simplifications. Real populations deviate from ideal (WF) populations in numerous ways, but many of these are captured in the concept of the N_e that is defined as the size of an ideal (WF) population that would experience the same rate of genetic change as the population under consideration (Wright 1931). Therefore, using estimates of this parameter as an input should capture the cumulative effects of the simplifying assumption. The largest challenge in this regard is that the N_e is difficult to estimate and if estimated based on temporal shifts in allele frequencies, this input will typically be based on the same data analyzed for outlier identification. However, the N_e estimation step can be seen as part of a model fitting procedure or if more independence of analysis steps are desired, alternative methods based on single sample methods can be used to estimate the N_e (recently reviewed in Luikart *et al.* 2010). In any case, the general insensitivity of the F_{temp} distribution to the magnitude of N_e (above a certain threshold) indicates robustness of conclusions regarding outliers despite uncertainty about the true N_e value. Only small N_e values changed the distribution significantly, but fortunately that represents the parameter range in which genetic methods for N_e estimation have highest precision and therefore should be least uncertain (Wang 2005; Palstra & Ruzzante 2008).

Depending on analysis objectives, the temporal differentiation in observed and simulated data can be quantified in several alternative ways. Here, we looked at the overall variance and therefore squared deviations from an overall mean because we were interested in detecting loci that showed elevated levels of variation in allele frequencies regardless of the direction of change or the specific sampling interval during which it occurred. The same simulated data could also be used to compute the more classical temporal F measures that is based on the squared difference between two temporal samples (see Waples 1989b), allowing for analysis of changes within specific shorter time periods. However, potential reductions in power and problems with correcting for multiple testing (pairwise non-independent comparisons) should be kept in mind.

The null distribution may also be simulated under a completely different model and set-up. We focused on standing genetic variation in a model only applicable to time frames short enough to reasonably ignore the effects of new mutations. For this purpose, we adopted a forward simulation approach because it allows for easier monitoring of changes in allele frequency in a population sampled at specific time intervals (Hoban *et al.* 2012). If temporal samples are collected further apart, e.g. with ancient DNA samples, it will be necessary to also model mutational processes and for such purposes it may be advantageous to base the neutral distribution on a coalescent simulator such as SerialSimCoal (Anderson *et al.* 2005) or Compass (Jakobsson 2009).

Our study shows that the Beaumont and Nichols (1996) approach is flexible enough to incorporate simulation results generated under a range of different models. The data presented here illustrate how the F_{temp} method offers a framework for a simpler and intuitively more appropriate approach for detecting outlier loci from temporal data than has previously been available. This method should make statistical inferences in relation temporal genome scan results more robust and easily interpretable.

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LITERATURE CITED

- Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: A workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics*, **9**, 323.
- Balding D (2003) Likelihood-based inference for genetic correlation coefficients. *Theoretical Population Biology*, **63**, 221–230.
- Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology*, **13**, 969–980.
- Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **263**, 1619–1626.
- Bollback JP, York TL, Nielsen R (2008) Estimation of 2Nes From Temporal Allele Frequency Data. *Genetics*, **179**, 497–502.
- Bourret V, O'Reilly PT, Carr JW, Berg PR, Bernatchez L (2011) Temporal change in genetic integrity suggests loss of local adaptation in a wild Atlantic salmon (*Salmo salar*) population following introgression by farmed escapees. *Heredity*, **106**, 500–510.
- Caballero A (1994) Developments in the prediction of effective population size. *Heredity*, **73**, 657–679.
- Cavalli-Sforza L (1966) Population structure and human evolution. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **164**, 362–379.
- Engen S, Ringsby TH, Saether B-E *et al.* (2007) Effective size of fluctuating populations with two sexes and overlapping generations. *Evolution*, **61**, 1873–1885.
- Excoffier L, Hofer T, Foll M (2009) Detecting loci under selection in a hierarchically structured population. *Heredity*, **103**, 285–298.
- Fisher RA, Ford EB (1947) The spread of a gene in natural conditions in a colony of the moth *Panaxia-dominula* L. *Heredity*, **1**, 143–174.
- Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, **180**, 977–993.
- Fraser DJ, Hansen MM, Østergaard S *et al.* (2007) Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. *Molecular Ecology*, **16**, 3866–3889.
- Gaines MS, Whittam TS (1980) Genetic changes in fluctuating vole populations: selective vs. nonselective forces. *Genetics*, **96**, 767–778.
- Goldringer I, Bataillon T (2004) On the distribution of temporal variations in allele frequency: Consequences for the estimation of effective population size and the detection of loci undergoing selection. *Genetics*, **168**, 563–568.
- Hansen MM, Meier K, Mensberg K-LD (2010) Identifying footprints of selection in stocked brown trout populations: a spatio-temporal approach. *Molecular Ecology*, **19**, 1787–1800.
- Hedrick PW, Ginevan ME, Ewing EP (1976) Genetic polymorphism in heterogeneous environments. *Annual Review of Ecology and Systematics*, **7**, 1–32.
- Hoban S, Bertorelle G, Gaggiotti OE (2012) Computer simulations: tools for population and evolutionary genetics. *Nature Publishing Group*, **13**, 110–122.
- Jakobsson M (2009) COMPASS: a program for generating serial samples under an infinite sites model. *Bioinformatics*, **25**, 2845–2847.
- Lewontin RC, Krakauer J (1973) Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. *Genetics*, **74**, 175–195.
- Lewontin RC, Krakauer J (1975) Letters to the editors: Testing the heterogeneity of F values. *Genetics*, **80**, 397–398.
- Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, **4**, 981–994.
- Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW (2010) Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conservation Genetics*, **11**, 355–373.
- Mueller L, Wilcox B, Ehrlich P, Heckel D (1985) A direct assessment of the role of genetic drift in determining allele frequency variation in populations of *Euphydryas editha*. *Genetics*, **110**, 495–511.
- Narum SR, Hess JE (2011) Comparison of FST outlier tests for SNP loci under selection. *Molecular Ecology Resources*, **11**, 184–194.
- Nei M, Maruyama T (1975) Letters to the editors: Lewontin-Krakauer test for neutral genes. *Genetics*, **80**, 395.
- Nunes VL, Beaumont MA, Butlin RK, Paulo OS (2010) Multiple approaches to detect outliers in a genome scan for selection in ocellated lizards (*Lacerta lepida*) along an environmental gradient. *Molecular Ecology*, **20**, 193–205.

- Orsini L, Spanier KI, De Meester L (2012) Genomic signature of natural and anthropogenic stress in wild populations of the waterflea *Daphnia magna*: validation in space, time and experimental evolution. *Molecular Ecology*, **21**, 2160–2175.
- Palstra FP, Ruzzante DE (2008) Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Molecular Ecology*, **17**, 3428–3447.
- Pérez-Figueroa A, García-Pereira MJ, Saura M, Rolán-Alvarez E, Caballero A (2010) Comparing three different methods to detect selective loci using dominant markers. *Journal of Evolutionary Biology*, **23**, 2267–2276.
- Poulsen N, Hemmer-Hansen J, Loeschcke V (2011) Microgeographical population structure and adaptation in Atlantic cod *Gadus morhua*. *Marine Ecology Progress Series*, **436**, 231–243.
- Rasmussen M, Li Y, Lindgreen S *et al.* (2010) Ancient human genome sequence of an extinct Palaeo-Eskimo. *Nature*, **463**, 757–762.
- Robertson A (1975) Remarks on the Lewontin-Krakauer test. *Genetics*, **80**, 396.
- Sandoval-Castellanos E (2010) Testing temporal changes in allele frequencies: a simulation approach. *Genetics Research*, **92**, 309–320.
- Stinchcombe JR, Hoekstra HE (2007) Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. *Heredity*, **100**, 158–170.
- Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 9440–9445.
- Storz JF (2005) Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology*, **14**, 671–688.
- Therkildsen NO, Nielsen EE, Swain DP, Pedersen JS (2010) Large effective population size and temporal genetic stability in Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 1585–1595.
- Wang J (2005) Estimation of effective population sizes from data on genetic markers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **360**, 1395–1409.
- Wang J (2009) A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Molecular Ecology*, **18**, 2148–2164.
- Wang JL, Whitlock MC (2003) Estimating effective population size and migration rates from genetic samples over space and time. *Genetics*, **163**, 429–446.
- Waples RS (1989a) Temporal variation in allele frequencies - testing the right hypothesis. *Evolution*, **43**, 1236–1251.
- Waples RS (1989b) A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics*, **121**, 379–391.
- Waples RS (1998) Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, **89**, 438–450.
- Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Notes*, **8**, 753–756.
- Watterson GA (1982) Testing selection at a single locus. *Biometrics*, 323–331.
- Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.
- Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–353.

FIGURE LEGENDS

Figure 1. Effect of sampling frequency and sample size on the distribution of F_{temp} . Shown are 0.025, 0.975 quantiles (solid lines) and median (dashed lines) of simulations using a Wright-Fisher population model of 500 individuals over 12 generations. In (a) sample sizes of 30 individuals were collected either in each generation, every other, every fourth generation or only at the beginning and end of the period. In (b) the population was sampled every fourth generation with sample sizes varying between 15 and 100 individuals.

Figure 2. Effect of generation length and N_e on the distribution of F_{temp} . Shown are 0.025, 0.975 quantiles (solid lines) and median (dashed lines) of simulations using a Wright-Fisher population model sampled with 30 individuals at four time points. In (a) the N_e is kept constant at 500, but the assumed generation length varies so that samples cover between 6 and 18 generations. In (b) and (c) the sampling covers 12 generations, but the N_e varies over generations starting at 500 (b) or the N_e is constant but of variable magnitude (c). Note the different scales on the y-axes.

Figure 3. Comparisons of results from the F_{temp} (a and c) and the Lositan method (b and d) applied to temporal data from an Atlantic cod population. The population was sampled at the beginning and end of a 12-generation period (a and b) or at 5 times over 6 generations (c and d). Each black dot represents single locus. Solid lines represent the 95% (grey) and 99% (black) confidence envelope of the simulated null distribution. Significant outliers after controlling the FDR to 5% are circled in red. Loci also significant outliers in BayeScan analysis are marked with blue circles (dark blue for outliers significant after FDR correction).

AUTHOR CONTRIBUTIONS BOX

NOT and EEN designed the research. NOT performed the research. NOT wrote the paper with substantial input from EEN.

Table 1. Overview of parameter settings for sensitivity analysis of the Ftemp model (see text for details)

Variable	t_s	n	ΔN_e	N_e	Result
t_s	[0, 2], [0, 4, 8, 12], [0, 2, 4, 6, 8, 10, 12], [0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]	30	Constant	500	Fig. 1a
n	[0, 4, 8, 12]	15, 30, 50, 100	Constant	500	Fig. 1b
GenLength	[0, 2, 4, 6], [0, 3, 6, 9], [0, 4, 8, 12], [0, 5, 10, 15], [0, 6, 12, 18]	30	Constant	500	Fig. 2a
ΔN_e	[0, 4, 8, 12]	30	Constant, fluctuating, decreasing	500	Fig. 2b
N_e	[0, 4, 8, 12]	30	Constant	50, 100, 500, 5000, 50000	Fig. 2c

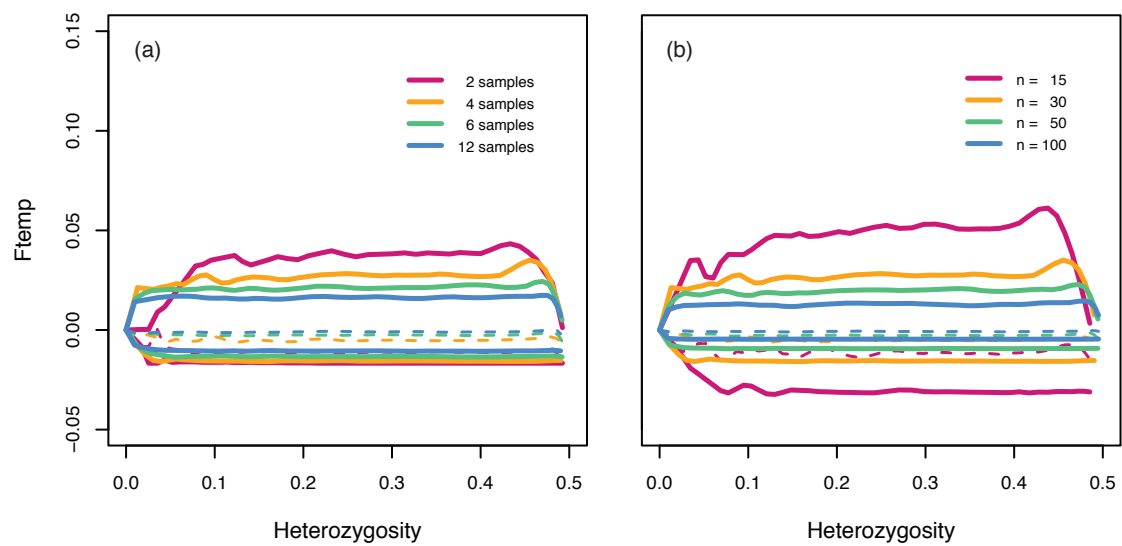


Fig. 1

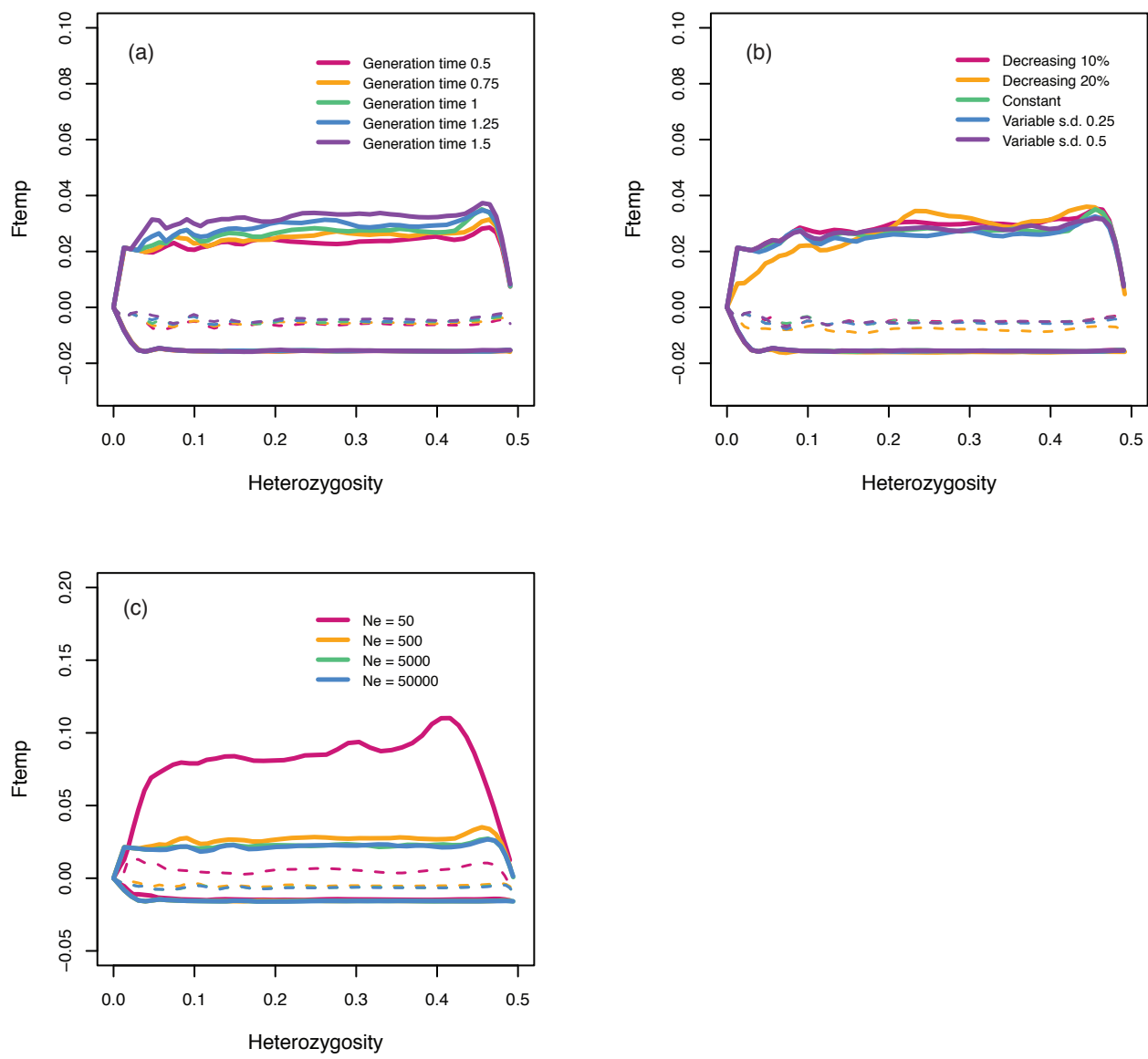


Fig. 2

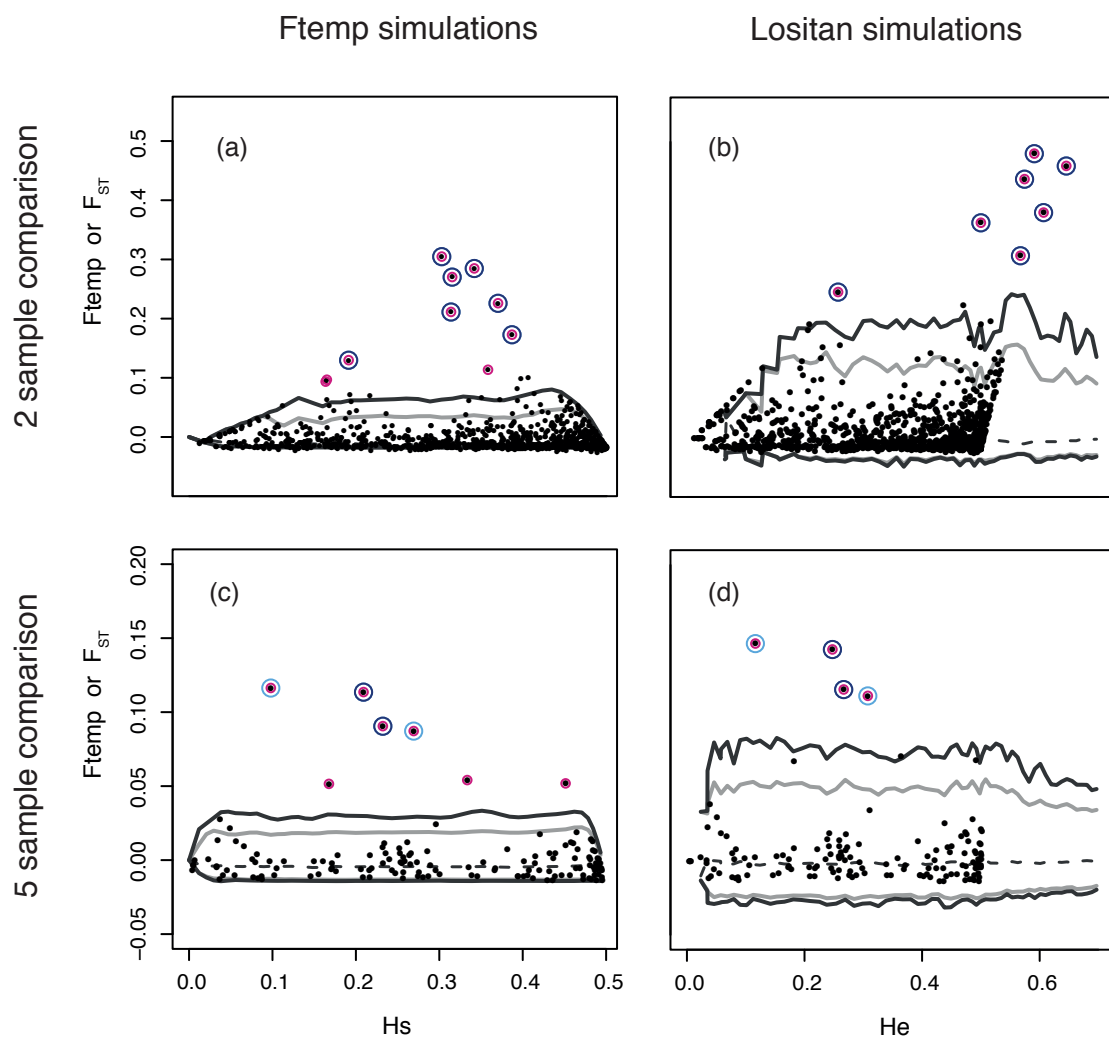


Fig. 3

List of additional manuscripts

Below is an overview of published and upcoming papers (not included in the thesis) resulting from additional work I have been involved in during my PhD.

De Wit, P., Pespeni, M. H., Ladner, J. T., Barshis, D. J., Seneca, S., Jaris, H., **Therkildsen, N. O.**, Morikawa, M., and Palumbi, S. R. 2012. The simple fool's guide to population genomics via RNA-Seq: an introduction to high-throughput sequencing data analysis. *Molecular Ecology Resources* 12: 1058-67.

Bothwell, H., Bisbing, S., Crawford, L., **Therkildsen, N. O.**, Alvarez, N., Holderegger, R., and Manel, S. *in press*. Identifying genetic variation of adaptive relevance in non-model species. *Conservation Genetics*.

Laugen, A. T., Engelhard, G. H., Whitlock, R., Arlinghaus, R., Dankel, D. J., Dunlop, E., Eikeset, A. M., Enberg, K., Jørgensen, C., Matsumura, S., Nusslé, S., Urbach, D., Baulier, L., Boukal, D. S., Ernande, B., Johnston, F. D., Mollet, F., Pardoe, H., **Therkildsen, N. O.**, Uusi-Heikkilä, S., Vainikka, A., Heino, M., Rijnsdorp, A. D., and Dieckmann, U. *in press*. Evolutionary impact assessment: accounting for evolutionary consequences of fishing in an ecosystem approach to fisheries management. *Fish and Fisheries*.

Hemmer-Hansen, J., Nielsen, E. E., **Therkildsen, N. O.**, Taylor, M. I., Ogden, R., Geffen, A., Bekkevold, D., Helyar, S., Pampoulie, C., Johansen, T., FishPopTrace Consortium, Carvalho, G. R. *in review*. A genomic island linked to ecotype divergence in Atlantic cod. *Molecular Ecology*



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